THE ROLE OF ENTEROCOCCUS FAECALIS SUGAR TRANSPORT IN EXPERIMENTAL COLITIS

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ABSTRACT

Ting-Jia Fan: The Role of *Enterococcus faecalis* Sugar Transport in Experimental Colitis
(Under the direction of Jonathan J. Hansen and R. Balfour Sartor)

Inflammatory bowel diseases (IBDs), which afflict 1.6 million people in the United States, are chronic, relapsing and immune-mediated intestinal disorders caused in part by aggressive T-cell-mediated immune responses to intestinal microbes in genetically susceptible individuals. However, relatively little is known about how intestinal inflammation affects the function of gut microbiota. We show that *Il10*$^{-/-}$ mice colonized with a simplified, defined microbial consortium that includes *Enterococcus faecalis* develop immune-mediated colitis. Transcription of two putative *E. faecalis* phosphotransferase systems (PTS) that import sugars into the bacterium is upregulated in colitis. We identify gluconate, ribose, and glucosamine as potential substrates for these PTS. The presence of these PTS is associated with altered intestinal microbial ecology and worsened colitis, therefore suggesting a role for *E. faecalis* sugar metabolism in colitis development. This work highlights the complexity of host-microbial-environmental interactions underlying colitis development and could enable the development of safe and effective treatments for IBDs.

I dedicate this work to my family

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LIST OF ABBREVIATIONS

AIEC	Adherent invasive *Escherichia coli*
BHI	Brain-heart infusion
CD	Crohn's disease
CD4	Cluster of differentiation 4
cDNA	Complementary DNA
CRISPR	Clustered regularly interspaced short palindromic repeats
DNA	Deoxyribonucleic acid
DSS	Dextran sulfate sodium
EDTA	Ethylenediaminetetraacetic acid
EI	Enzyme I
EII	Enzyme II
ELISA	Enzyme-linked immunosorbent assay
Erm	Erythromycin
gDNA	Genomic DNA
GF	Germ-free
GWAS	Genome-wide association studies
HPr	Heat-stable phosphoryl carrier protein
IBDs	Inflammatory bowel diseases
IL	Interleukin
mRNA	Messenger RNA

NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information
NMR	Nuclear magnetic resonance
PCR	Polymerase chain reaction
PTS	Phosphotransferase system
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
SPF	Specific pathogen free
TNF	Tumor necrosis factor
Tris	Tris(hydroxymethyl)aminomethan
tRNA	Transfer RNA
UC	Ulcerative colitis
WT	Wild-type

CHAPTER 1: INTRODUCTION

1.1 Inflammatory bowel diseases (IBDs)

Inflammatory bowel diseases (IBDs), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic, relapsing immune-mediated inflammatory disorders of the intestines (Sartor, 2006; Sartor & Wu, 2017). CD is a condition of chronic inflammation in any location of the gastrointestinal tract and UC is a chronic gastrointestinal disorder that is limited to the large bowel (Khor, Gardet, & Xavier, 2011). An estimated 1.6 million people in the United States currently have IBDs and as many as 70,000 new cases of IBD are diagnosed each year (Loftus, 2016; Shivashankar, Tremaine, Harmsen, & Loftus, 2017). An estimated direct and indirect annual medical cost of IBDs in United States is between $14.6 and $31.6 billion (Mehta, 2016). The incidence and prevalence of IBDs has steadily increased in developing countries and has been attributed to these country's adoption of a Westernized lifestyle (M'Koma, 2013). Currently, IBDs cannot be cured medically and surgical treatments for CD offer only temporary relief (Hansen & Sartor, 2015). Most effective medical therapies for IBDs are immune suppressing and therefore associated with potentially toxic side effects (Baumgart & Sandborn, 2012; Ordás, Eckmann, Talamini, Baumgart, & Sandborn, 2012). Therefore, alternative new and effective treatments that do not cause significant sides effects are needed.

1.2 Pathogenesis of IBDs

Although the exact causes of IBDs are unknown, a prevailing hypothesis is that the etiology of IBDs is a combination of genetic susceptibility, dysregulated immune responses,

environmental triggers and altered resident intestinal microbes (Sartor, 2006). The large-scale genome-wide association studies (GWAS) have shown more than 200 genetic loci that are associated with IBDs and many of these IBD risk loci are in gene regions that encode proteins involved in innate/adaptive immune responses, mucosal barrier function and bacterial clearance (Jostins et al., 2012; Liu et al., 2015).

In addition to GWAS, immunological studies also implicate maladaptive immune responses in the pathogenesis of IBDs. Activated macrophages and dendritic cells in the lamina propria are increased in IBD patients (Sartor, 2006; Sartor & Wu, 2017), and most pro-inflammatory cytokines and chemokines, such as interleukin (IL)-1ß, tumor necrosis factor (TNF), IL-6, IL-8, IL-12, and IL-18, are also increased in both CD and UC (Sartor, 2006). Dysfunctional immune regulation in IBDs results in innate/adaptive immune cells, such as macrophages, dendritic cells, neutrophils and T cells, being recruited into the inflamed intestine and then releasing pro-inflammatory cytokines and chemokines prolonging inflammation (Sartor, 2006; Sartor & Hoentjen, 2005).

Some studies show that environmental factors also contribute to the pathogenesis of IBDs. For instance, infection, diet, antibiotic and nonsteroidal anti-inflammatory drug usage, stress, and smoking may initiate or perpetuate dysregulated immune responses (Loftus, 2004; Sartor, 2006). Altered resident intestinal microbial composition, also known as microbial dysbiosis, is associated with IBDs (Bibiloni, Mangold, Madsen, Fedorak, & Tannock, 2006; Sartor & Wu, 2017). Although there is no direct evidence that intestinal microbiota cause human IBDs, several studies provide indirect evidence that intestinal microbes may play a role in development of IBDs (Sartor, 2008; Sartor & Wu, 2017). For example, fecal stream diversion for 3 to 6 months prevents Crohn's inflammation; however, inflammation recurs after restoration of fecal flow

(D'Haens et al., 1998; Rutgeerts et al., 1991). In summary, our current understanding of molecular mechanisms of IBDs pathogenesis is that environmental triggers initiate and perpetuate aggressive T-cell-mediated immune responses to specific intestinal microbiota components in genetically susceptible individuals (Hansen, 2015; Sartor & Wu, 2017).

1.3 Intestinal microbiota and IBDs

The distal-intestinal tract harbors the highest concentration of resident microbial communities, known as intestinal microbiota, in the human body and provides an interface for intestinal microbiota to interact with the host through complex molecular cross-talk (Montalto, D'onofrio, Gallo, Cazzato, & Gasbarrini, 2009). At birth, the gastrointestinal tract is quickly colonized with maternal or environmental microbes. The intestinal microbiota increases in complexity and stability over the first two years of life before maturation into an adult state (Koenig et al., 2011; S. Subramanian et al., 2014). The majority of the normal adult enteric microbiota belong to bacterial phyla Firmicutes and Bacteroidetes, followed by Proteobacteria, Actinobacteria, Verrucomicrobia and Fusobacteria that are present in lesser abundance (Eckburg et al., 2005). The intestinal microbiota is commonly referred to as a hidden “organ” because they are important for normal intestinal development, homeostasis and protection against pathogen infection. Moreover, intestinal microbiota also impact host metabolism, physiology, nutrition and immune function (Guinane & Cotter, 2013).

IBDs are associated with altered composition of specific groups of intestinal bacteria (Darfeuille-Michaud et al., 2004; Lepage et al., 2011; Q. Li, Wang, Tang, Li, & Li, 2012; Martinez Medina, Aldeguer, Gonzalez Huix, Acero, & Garcia Gil, 2006; Sokol et al., 2008). For example, IBDs are associated with decreased microbial biodiversity/richness and decreased compositional stability. Recently, a large microbiome study in human IBDs found that mucosal

biopsies from pediatric patients with new onset CD were more likely to have increased abundance of Enterobacteriaceae, Pasteurellaceae, Veillonellaceae and Fusobacteriaceae, and decreased abundance in Erysipelotrichales, Bacteroidales and Clostridiales, which findings are consistent with the prior studies (Gevers et al., 2014). Interestingly, in this same cohort, microbial compositional shifts in biopsies from inflamed ilea were also observed in biopsies of non-inflamed ilea, suggesting that microbiota changes may be the cause rather that the result of inflammation (Haberman et al., 2014). Despite recent advanced molecular sequencing technologies to characterize complex microbial communities, direct evidence that microbes cause human IBDs is still lacking.

1.4 The *Il10*$^{-/-}$ mouse model of chronic, immune-mediated colitis

The most direct evidence that intestinal microbes cause IBDs comes from experiments in rats and mice, including interleukin-10-deficient (*Il10*$^{-/-}$) mice. IL-10 is an anti-inflammatory cytokine that limits the innate/adaptive immune response to pathogens and prevents damage to host tissues, and is therefore important to the maintenance of mucosal homeostasis (Saraiva & O'Garra, 2010). *Il10*$^{-/-}$ mice raised in conventional housing facilities develop spontaneous chronic intestinal inflammation characterized by body weight loss, immune cells infiltration and distortion of crypt architecture (Kühn, Löhler, Rennick, Rajewsky, & Müller, 1993). However, the chronic intestinal inflammation is attenuated in *Il10*$^{-/-}$ mice raised under specific pathogen free (SPF) conditions and is absent in *Il10*$^{-/-}$ mice raised in sterile, germ-free (GF) conditions (Berg et al., 1996; Sellon et al., 1998).

Selective colonization of mice using defined bacterial strains, known as gnotobiotics, demonstrates that specific resident microbial taxa exhibit varying abilities to induce intestinal inflammation in these mice. For example, *Il10*$^{-/-}$ mice develop colitis when mono-colonized with

a single strain of adherent invasive *Escherichia coli* (AIEC), NC101, but not when colonized with a non-AIEC *E. coli* strain, K12 (Ellermann et al., 2015; Kim, Tonkonogy, Jarvis, Darfeuille-Michaud, & Sartor, 2008). Similarly, GF *Il10*$^{-/-}$ mice also develop colitis when colonized with a defined consortium of non-pathogenic bacterial species, but the inflammation is less severe compared with SPF-colonized GF *Il10*$^{-/-}$ mice (Eun et al., 2014; Sellon et al., 1998). Importantly, GF wild-type (WT) mice do not exhibit any histological inflammation when mono-colonized with NC101 or colonized with the defined consortium of bacterial species (Eun et al., 2014; Patwa et al., 2011). Taken together, these studies highlight the power of gnotobiotic animal experiments to demonstrate that bacteria cause disease and to decipher some of the complex host-microbe interactions. Gnotobiotic animals also provide a more clinically-relevant environment than lab culture media to study interactions between different microbes in the intestine. In this work, we use gnotobiotic *Il10*$^{-/-}$ mice to study inter-species bacterial interactions in the gut and the resulting impact on experimental colitis.

1.5 *Enterococcus faecalis*

In addition to *E. coli*, other bacterial species have been implicated in human IBDs and experimental colitis. For instance, *Enterococcus* species are more abundant in mucosal biopsies from IBDs patients vs. controls (Chen et al., 2014; De Cruz et al., 2015). Specifically, *Enterococcus faecalis*, a non-motile, Gram-positive, facultative anaerobe, is more abundant in feces from CD patients vs. healthy controls (Zhou et al., 2016). In mono-colonized *Il10*$^{-/-}$ mice, *E. faecalis* induces a slower-onset, distal colon-predominant inflammation, which progresses to severe distal colitis and duodenal inflammation at later time points (Kim et al., 2005). *E. faecalis* and *E. coli* dual-colonized *Il10*$^{-/-}$ mice develop more rapid onset and aggressive pancolitis and duodenal inflammation than mono-colonized mice (Kim, Tonkonogy, Karrasch, Jobin, & Sartor,

2007). These findings suggest that *E. faecalis* causes experimental colitis and may contribute to human IBDs.

1.6 Colitis-associated alterations in intestinal bacterial composition and transcription

Although intestinal microbiota dysbiosis correlates with IBDs, how factors in the intestine affect gut microbial community structure and function is still unclear. These environmental factors include diet, antibiotics, and intestinal inflammation. Feeding pediatric Crohn's disease patients exclusively with a liquid formula diet induces remission (Dunn et al., 2016; Lewis et al., 2015) and is associated with increased intestinal microbial diversity (Dunn et al., 2016). Also, inflammation, antibiotic exposure and diet independently affect different microbial taxa in Crohn's disease patients (Lewis et al., 2015).

In addition to altered gut bacterial composition, IBDs are also associated with changes in gut bacterial function. We have previously shown that host inflammation affects luminal bacterial functions, which in turn impacts the course of disease. For example, stress response gene expression is upregulated in luminal bacteria from GF *Il10*$^{-/-}$ mice mono-colonized with the *E. coli* NC101 and the presence of two of these stress response genes results in decreased colitis (Patwa et al., 2011; Tchaptchet et al., 2013).

1.7 Carbohydrates and IBDs

With the understanding that intestinal bacterial likely contribute to the pathogenesis of IBDs, manipulating the gut bacteria composition and function for therapeutic purposes is an attractive approach. While several studies have shown that dietary treatments change gut bacterial composition in the host (Lewis et al., 2015; Wu et al., 2011), there is no direct evidence to suggest that specific diets can prevent or cure IBDs. Recently, several studies have shown that nutritional approaches, including consuming specific dietary carbohydrates (Kakodkar, Farooqui,

Mikolaitis, & Mutlu, 2015; Knight-Sepulveda, Kais, Santaolalla, & Abreu, 2015; Olendzki et al., 2014), reduce some symptoms of IBDs.

Measuring fecal concentrations of carbohydrates in patients with IBDs may also help clarify the role of carbohydrates in the pathogenesis of IBDs. Using 1H NMR spectroscopy of fecal extracts from UC patients and healthy control subjects, investigators found increased taurine, cadaverine, choline and glucose levels in UC compared with controls (Le Gall et al., 2011). Therefore, though not yet tested, it is possible that elevated glucose in the colon lumen of UC patients could cause dysbiosis and impact disease severity. Other investigators used a similar technique to measure fecal metabolites in the dextran sulfate sodium (DSS)-induced colitis mouse model (Hong et al., 2010). They found increased monosaccharides, glucose, xylose, and trimethylamine in the feces of mice with DSS colitis compared with healthy controls. While these studies correlate the presence of certain fecal carbohydrates with colitis, how these inflammation-associated changes in luminal carbohydrates affect the gut microbiota and perpetuate inflammation is unknown.

1.8 Phosphotransferase system (PTS)

Among the several pathways that bacteria employ to acquire and metabolize environmental carbohydrates, the phosphotransferase system (PTS) is commonly used by obligate and facultative anaerobes such as *E. faecalis*. There are 39 putative PTS in *E. faecalis* OG1RF based on NCBI genome annotation. The PTS consists of two cytoplasmic components, phosphoenolpyruvate (PEP)-dependent kinase enzyme I (EI) and heat-stable phosphoryl carrier protein (HPr), that are general PTS components involved in all PTS pathways. Unlike general PTS components, PTS enzyme II (EII) complexes are thought to be specific for each sugar substrate and consist of IIA, IIB, IIC, and sometimes IID proteins and/or protein domains

(Galinier & Deutscher, 2017). Deletion of any of the EII components of a given EII complex typically inhibits import of the cognate sugar substrate for that complex. PTS couples the transport of sugars across the cytoplasmic membrane with their phosphorylation so that these sugars can be used as carbon sources for metabolism (Deutscher et al., 2014). The PTS also serves as a signal transduction system that monitors carbohydrate utilization based on the flux of phosphate through the pathway and can also directly regulate the metabolism of non-PTS sugars (Deutscher et al., 2014).

In addition to their influence on sugar metabolism, PTS can also affect the virulence of some bacterial pathogens. For instance, three different pathogenic bacterial strains (*Salmonella typhimurium, Staphylococcus aureus*, and *Haemophilus influenzae*) deficient in EI exhibit attenuated virulence in a mouse model of peritonitis compared with parental strains (Kok, Bron, Erni, & Mukhija, 2003). In another study, Hondorp, *et al*. show that the virulence regulator Mga in group A *Streptococcus* (GAS) contains a phosphotransferase system regulatory domain that can be phosphorylated in vitro by the EI/HPr (Hondorp et al., 2013). Mutations in this regulatory domain of Mga are associated with attenuated invasive GAS skin disease in a mouse model. This study implicates PTS-mediated phosphorylation of Mga in bacterial virulence gene expression (Hondorp et al., 2013). Whether the presence of environmental carbohydrates impacts PTS-mediated virulence gene expression remains to be determined.

1.9 Thesis Aims

While the role of gut bacteria in the pathogenesis of IBDs and experimental colitis is becoming increasingly clear, large knowledge gaps exist. For instance, how does intestinal inflammation affect gut bacterial transcription in more complex microbial communities? What is the role of bacterial carbohydrate metabolism in the pathogenesis of intestinal inflammation?

How might bacterial species interact with one another in the intestine to influence inflammation? In the present work, we hope to begin to answer these host-microbial-environmental interaction questions and hope by answering these questions could guide the development of new strategies and potentially novel treatment options for IBD patients.

CHAPTER 2: MATERIALS AND METHODS

2.1 Animals and bacterial species

Germ-free WT and *Il10*$^{-/-}$ mice on the 129S6/SvEv background were obtained from the National Gnotobiotic Rodent Resource Center (UNC-Chapel Hill). All mice were housed in sterilized cages and provided with sterilized food and drinking water ad libitum. Mice were colonized at 8 to 16 weeks of age with eight IBD or experimental colitis-related intestinal bacterial strains, namely, *Escherichia coli* NC101 (Kim et al., 2005), *Lactobacillus rhamnosus* GG, *Enterococcus faecalis* OG1RF, *Bacteroides thetaiotaomicron* VPI-5482, *Bacteroides vulgatus* ATCC 8482, *Faecalibacterium prausnitzii* A2-165, *Bifidobacterium longum* subsp. Infantis ATCC 15697, and *Ruminococcus gnavus* VPI C7-9 (ATCC 29149), by oral gavage. Animal protocols were performed in accordance with American Association for Laboratory Animal Care standards and were approved by the UNC Institutional Animal Care and Use Committee.

2.2 Histological analysis

In WT or *Il10*$^{-/-}$ mice, inflammation in formalin fixed, hematoxylin and eosin stained sections of cecum, proximal colon, mid-colon, and distal colon was quantified in a blinded fashion and summed together as described previously (Patwa et al., 2011).

2.3 Microbial RNA isolation and RNA-sequencing Analysis

Total bacterial RNA was isolated from the cecal content of wild type and *Il10*$^{-/-}$ mice (n = 5 mice/group) using a combination of bead beating and phenol:chloroform extraction as described

previously (Faith, McNulty, Rey, & Gordon, 2011). To deplete total microbial community RNA of 16S, 23S, 5S rRNA and tRNA species prior to synthesis of cDNA with random hexanucleotide primers, each fecal RNA preparation was subjected to column-based size-selection and hybridization to custom biotinylated oligonucleotides directed at conserved regions of bacterial rRNA genes present in human gut communities, followed by streptavidin-bead based capture of the hybridized RNA sequences (Rey et al., 2010). Barcoded Illumina sequencing libraries were prepared from double-stranded cDNA generated from each of the mRNA enriched samples, and the 10-sample pool was sequenced on one lane of an Illumina HiSeq2000 sequencer. After barcode removal, each of the RNA-seq data sets was composed of 38-nucleotide-long reads (3.57 ± 0.8 × 10^7/mRNA reads per sample). Transcript abundances were normalized for each of the 8 species in each sample to reads per million per kilobase (RPMK). For conserved protein domain analysis, RNA-Seq reads aligned to the genomes of the eight bacterial strains were categorized by conserved protein domain based on the NCBI conserved protein domain database on August 15, 2012. We then performed gene set enrichment analysis (GSEA, Broad Institute, http://software.broadinstitute.org/gsea/index.jsp) to determine conserved domains that were differentially enriched in a given mouse genotype (A. Subramanian et al., 2005).

2.4 Enzyme-Linked ImmunoSorbent Assays (ELISA)

Segments of proximal colon (2-3cm long) were washed, weighed, minced, and cultured for 22 hours as described previously after which culture supernatants were stored at -80 ºC (Patwa et al., 2011). Mouse IL-12/23 p40 in culture supernatants was measured by ELISA as described previously (Patwa et al., 2011).

2.5 Bacterial gDNA isolation

Luminal gDNA was isolated by incubating cecal or colonic content in 500 µL lysis buffer (200 mM NaCl, 100 mM Tris pH 8.0, 20 mM EDTA, 20 mg/mL lysozyme) at 37 ºC for 30 min, then adding 30 µL of 20% SDS and 30 µL proteinase K (20 mg/mL), and incubating at 60 ºC for 30 min. Three hundred µL of 0.1 mm glass beads (Biospec) and 500 µL of phenol:chloroform:isoamyl alcohol (25:24:1, pH 8) were added and then samples were lysed in the TeSeE Precess48 bead beater (Bertin Technologies) for 3 rounds of 90 sec at 6200 rpm. After centrifugation, the aqueous layer was extracted with another 500 µL of phenol:chloroform:isoamyl alcohol followed by 500 µL chloroform. The DNA in the aqueous phase was precipitated with ethanol, resuspended in Tris-EDTA buffer, and then further purified using DNeasy Mini Spin Columns according to the manufacturer's instructions (Qiagen, Valencia, CA).

2.6 Quantitative real-time PCR

For quantitative PCR, species-specific oligonucleotide primers (detail sequence see Table 2.1) were used to target bacterial species-specific hypervariable regions of the 16S rRNA gene. Twenty nanograms of bacterial gDNA was added to 8 µL of iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA) and 0.4 µM of the forward and reverse primers as indicated in table S1 and placed in the Applied Biosystems QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA) running universal conditions. Data were analyzed using the deltaCt method with reference to total-bacteria 16S primers.

2.7 Generation of *E. faecalis* EIIA^{11616} and EII^{gnt} mutant strains

Deletion of EIIA^{11616} and EII^{gnt} genes from *E. faecalis* OG1RF chromosome was accomplished by allelic replacement using the temperature-sensitive shuttle vector pJRS233

(Perez-Casal, Price, Maguin, & Scott, 1993). Deletion alleles were constructed by PCR amplification of upstream (US) and downstream (DS) DNA fragments (~1,000 bp each) on either side of $EIIA^{11616}$ or EII^{gnt} genes using primer pairs (Table 2.2) PTSIIA-1.A-EcoR1/PTSIIA-1.B-BamHI (US of $EIIA^{11616}$), PTSII-2.A-BamHI/PTSIIA-2.B-NcoI (DS of $EIIA^{11616}$) or uPTSXmaF/uPTSXbaR (US of EII^{gnt}), dPTSXbaF/dPTSSacR (DS of EII^{gnt}). These PCR products were sequentially cloned into pJRS233 shuttle vector, using standard cloning techniques to create pJH190 (for $EIIA^{11616}$ deletion mutant) and pJH192 (for EII^{gnt} mutant). Deletion constructs were then transformed into *E. faecalis* OG1RF by electroporation and the transformants were selected at 30 °C on erythromycin (Erm). Chromosomal integrates were selected by growing at 42 °C in the presence of Erm. Selection for excision of the integrated plasmid by homologous recombination was accomplished by growing the bacteria at 30 °C in the absence of Erm. Erm-sensitive colonies with double-crossover events that integrated deletion alleles were selected by PCR screening. Deletions were confirmed by PCR and DNA sequencing.

2.8 Bacterial growth assay

Parental or mutant bacterial overnight cultures in BHI were diluted to 1.0 optical density at 600 nm (OD_{600}) and added (1:100 ratio) to M9 based minimal media supplemented with essential and non-essential amino acids (Invitrogen, Carlsbad, CA) and MEM vitamin solution (Invitrogen, Carlsbad, CA) and the indicated carbohydrate source. The cultures were incubated at 37° C in aerobic or anaerobic conditions. At the specified time points, an aliquot of the culture was taken for OD_{600} measurement.

2.9 Biolog™ screen

Biolog Phenotype Micro Arrays™ PM1-PM5 on *E. faecalis* and ΔEIIA^{11616} were performed by Biolog, Inc (Hayward, CA). PM1 contains primarily carbohydrate and carboxylate substrates, whereas PM2 through PM5 contain individual L-amino acids and most dipeptide combinations, for a total of 367 substrate nutrients. Parental and mutant EIIA^{11616} bacteria picked from colonies grown on agar plates were diluted to the same concentrations using proprietary minimal media and added to PM1-PM5. A proprietary redox dye was added, and the plates were placed in the OmniLog® automated incubator/reader for 22 hours.

2.10 Statistical analysis

P values were calculated using two-tailed Student's t test when two experimental groups were compared. All data are presented as the mean ± SEM.

2.11 Tables

Table 2.1 Species-specific 16S-targeted Primers for Quantitative PCR.

Target bacterial species	Primer sequence (5’ -> 3’)
Escherichia coli NC101	F: 5'- CGTGTTGTGAAATGTTGGGTTAA -3' R: 5'- CCGCTGGCAACAAAGGATAA -3'
Lactobacillus rhamnosus GG	F: 5'- GAGAAGAATGGTCGGCAGAGTAA -3' R: 5'- CACGTAGTTAGCCGTGGCTTT -3'
Enterococcus faecalis OG1RF	F: 5'- CGCGGTGCATTAGCTAGTTG -3' R: 5'- TCACCCTCTCAGGTCGGCTAT -3'
Bacteroides thetaiotaomicron VPI-5482	F: 5'- CAGTGTCAGTTGCAGTCCAGTGA -3' R: 5'- GTGTAGCGGTGAAATGCTTAGATATC -3'
Bacteroides vulgatus ATCC 8482	F: 5'- GGTCGCCATCGGCCTTA -3' R: 5'- CTAGCTACAGGCTTAACACATGCAA -3'
Faecalibacterium prausnitzii A2-165	F: 5’- CCCTTCAGTGCCGCAGT -3’ R: 5’- GTCGCAGGATGTCAAGAC -3’
Bifidobacterium longum subsp. Infantis ATCC 15697	F: 5'- GCAACGCGAAGAACCTTACC -3' R: 5'- CGCCCCGAAGGGAAAC -3'
Ruminococcus gnavus VPI C7-9 (ATCC 29149)	F: 5'- AACGTACTCCCCAGGTGGAAT -3' R: 5'- CGGGTGGCAAAGCCATT -3'
All bacteria universal	F: 5’- GTGSTGCAYGGYTGTCGTCA -3’ R: 5’- ACGTCRTCCMCACCTTCCTC -3’

Table 2.2 Primer sequence for Cloning.

Primer name	Primer sequence (5’ -> 3’)
PTSIIA-1.A-EcoR1	5'- tatagaattcTCAAGTAAGCCTTTAATGGTG -3'
PTSIIA-1.B-BamHI	5'- gcgcggatccAGAAGAAGAATTTTAGGAGG -3'
PTSIIA-2.A-BamHI	5'- tataggatccTCACTTACTTCTTTACATAGC -3'
PTSIIA-2.B-NcoI	5'- gcgcccatggTGGAACGTCAAGAAACGAGG -3'
Pts2AXma	5'- tatacccgggTCAAGTAAGCCTTTAATGGTG -3'
Pts2BXma	5'- gcgccccgggTGGAACGTCAAGAAACGAGG -3'
uPTSXmaF	5'- tcccgggAATATAGGTCAACCCCATTGCTC -3'
uPTSXbaR	5'- ctctagaGAATGTAGTAAAGGGTGAAAGCAATG -3'
dPTSXbaF	5'- ctctagaAGTTCCTCCTTCTTATCCTTGGTT -3'
dPTSSacR	5'- tccgcggGGTGCCGTCACTTTTCAAGGAA -3'

CHAPTER 3: RESULTS

3.1 Inflammation is associated with altered bacterial transcription in mice colonized with complex microbiota

Previous work has identified colitis-associated transcriptome profiles of bacteria from mono-colonized mice (Arthur et al., 2014; Patwa et al., 2011). To address the effects of intestinal inflammation on commensal microbes in a more complex luminal environment, we selectively colonized GF WT and *Il10*$^{-/-}$ mice with a mixture of eight bacterial strains representing each of the major phyla found in the mammalian colon. We chose these particular strains because the genomic sequences are known, which facilitates the analysis of transcriptional data sets, and because many of them have been implicated in human IBDs or experimental colitis.

We found that histological inflammation was greater in *Il10*$^{-/-}$ mice compared to WT mice at both 5 and 10 weeks' post-colonization and that inflammation in *Il10*$^{-/-}$ mice was greater at 10 weeks than at 5 weeks (Figure 3.1A). Similar to the histological inflammation, we detected increased IL-12/23 p40 secretion by colon tissue explants from *Il10*$^{-/-}$ vs. WT mice colonized with the bacterial mixture for both 5 and 10 weeks (Figure 3.1B).

We next wanted to determine whether colitis in the 10-week colonized mice is associated with altered bacterial function. To assess function, we first measured global transcriptional activity of each bacterial strain using microbial RNA-Sequencing (RNA-seq) technology. The largest number of ≥1.5-fold differentially expressed genes is found in *E. faecalis* (Table 3.3 and 3.4) followed by *R. gnavus*, *B. vulgatus*, and *B. thetaiotaomicron* (Figure 3.2). We performed a

principal components analysis and found that the bacterial meta-transcriptome in *Il10*$^{-/-}$ mice is different from that in WT mice (Figure 3.3). We next used conserved domain analysis to determine which of the known protein domains are significantly enriched in *Il10*$^{-/-}$ and WT mice. In bacteria from *Il10*$^{-/-}$ mice with colitis, we detected enrichment of transcripts that encode protein domains important in transcription/translation, cell wall synthesis, anaerobic metabolism, carbohydrate metabolism, stress responses, and DNA repair (Table 3.1). In bacteria from healthy WT mice, we detected enrichment of transcripts that encode protein domains important in redox potential maintenance, iron uptake, cation transport, and cell signaling (Table 3.2). Four out of 10 enriched conserved protein domains we detected from *Il10*$^{-/-}$ mice are involved with carbohydrate metabolism. This suggests that the carbohydrate landscape in the lumen of the inflamed colon differs from that in the non-inflamed colon and/or that metabolism of carbohydrates by luminal bacteria is impacted by colitis.

Since the largest number of differentially expressed genes in our model system is found in *E. faecalis* and colitis impacts bacterial carbohydrate metabolism in general, we predicted that many of the differentially expressed genes in *E. faecalis* would involve carbohydrate metabolism. Indeed, the *E. faecalis* PTS operon including OG1RF_11616 was the most highly upregulated set of genes in *E. faecalis* during colitis (Table 3.3). Another *E. faecalis* PTS operon including OG1RF_12399 was also highly upregulated during colitis (Table 3.3). PTS couple the transport of sugars across the cytoplasmic membrane with their phosphorylation so that they can be used as a carbon source for metabolism. PTS also serve as signal transduction systems that monitor carbohydrate utilization and control other carbohydrate metabolic processes based on the flux of phosphate through the pathway. We hypothesized that the carbohydrate substrates for

these two *E. faecalis* PTS operons may contribute to experimental colitis and therefore sought to identify them.

3.2 The substrates for PTS EIIA^{11616} and EII^{12399}

To determine the substrates of the PTS encoded by the OG1RF_11616 and OG1RF_12399 operons, we first created two mutant strains of *E. faecalis* (*E. faecalis* ΔEIIA^{11616} and *E. faecalis* ΔEII^{12399}). We then tested growth of the mutant and parental strains in minimal media containing fructose, mannose or sorbose as the sole carbon source since these sugars are putative candidates based on the NCBI annotation of these operons. There was no growth difference between parental and mutant *E. faecalis* in M9 minimal media supplemented with fructose or mannose (Figure 3.4A and B), and none of strains grew in media containing sorbose (Figure 3.4C). Therefore, we conclude that fructose, mannose, and sorbose are not substrates for the PTS encoded by either of these PTS operons.

We then performed a dedicated sequence alignment of genes in the OG1RF_11616 operon with bacterial genes in the NCBI prokaryotic database and found sequence similarity between certain genes in this operon and genes in the database that were annotated as glucosamine-fructose-6-phosphate aminotransferase. Therefore, we repeated the growth assays in minimal media supplemented with glucosamine and found that mutant *E. faecalis* ΔEIIA^{11616} has a slight growth deficit compared to parental strain (Figure 3.4D). Therefore, the PTS encoded by the OG1RF_11616 operon facilitates, but is not necessary for, glucosamine utilization.

Because our targeted approach did not clearly establish the substrate used by the PTS encoded by the OG1RF_11616 operon, we performed a high-throughput screen by growing *E. faecalis* ΔEIIA^{11616} and parental *E. faecalis* OG1RF on a large variety of substrates in the Biolog Phenotype Microarray System, which measures the ability of bacteria to use substrates to

produce reducing substances including NADH. The screen showed that parental and mutant *E. faecalis* ΔEIIA^{11616} strains had similar metabolic activity on most substrates. However, potential differences in metabolic activity between the two strains were detected for five substrates: gluconic acid, glucosaminic acid, frucotose-6-phosphate, inosine and dulcitol (Figure 3.5). To determine whether the OG1RF_11616 operon encodes proteins that permit *E. faecalis* to use these five substrates for growth, we tested whether *E. faecalis* ΔEIIA^{11616} could grow in minimal media supplemented with these carbohydrates. We found that both strains grow similarly in glucosaminic acid and frucotose-6-phosphate, and neither strain grows in dulcitol. Interestingly, mutant *E. faecalis* ΔEIIA^{11616} grows better than the parental strain in gluconic acid. Neither strain grows efficiently in inosine-supplemented media, but the parental *E. faecalis* OG1RF does have a slight growth advantage over *E. faecalis* ΔEIIA^{11616} (Figure 3.6B).

In addition to the five substrates identified as strong candidates in the Biolog screen, we also observed weaker effects of the nucleosides thymidine, uridine, and adenosine on OG1RF_11616-mediated metabolism in the Biolog screen (Figure 3.5). Neither *E. faecalis* OG1RF nor *E. faecalis* ΔEIIA^{11616} grows efficiently in adenosine or uridine, but *E. faecalis* OG1RF does have a slight growth advantage (Figure 3.6). Since ribose is a shared component of nucleosides, we predicted that the OG1RF_11616 operon might encode genes that metabolize ribose. To test this, we performed growth assays in minimal media plus ribose and found that parental *E. faecalis* OG1RF grows slightly better than *E. faecalis* ΔEIIA^{11616} (Figure 3.6A). Similar to the data for glucosamine, these data suggest that gene products of the OG1RF_11616 operon facilitate, but are not required for, ribose metabolism.

Without having clearly identified the substrate for the PTS encoded by the OG1RF_11616 operon, we then turned our attention to identifying the substrate for the PTS encoded by the

OG1RF_12399 operon. We found that EII^{12399} shares amino acid sequence similarity with the *E. faecalis* V583 EF3136 gene, which has been suggested by others to transport gluconate. To test whether the proteins encoded by the OG1RF_12399 operon also transport gluconate, we grew parental and mutant *E. faecalis* ΔEII^{12399} in M9 minimal media supplemented with sodium gluconate as a sole carbon source. Parental *E. faecalis* OG1RF grows efficiently in gluconate-containing media, but *E. faecalis* ΔEII^{12399} fails to grow thus confirming that the PTS encoded by the OG1RF_12399 operon transports gluconate and is necessary for gluconate utilization by *E. faecalis* OG1RF (Figure 3.7). Therefore, we designate PTS EII^{12399} as PTS EII^{gnt}.

3.3 *E. faecalis* PTS genes are associated with increased inflammation in *Il10*$^{-/-}$ mice

Though the effects of inflammation on luminal gluconate, ribose, and glucosamine concentrations in the intestine are unknown, we sought to determine whether *E. faecalis* genes that encode proteins that participate in metabolism of these carbohydrates affect the severity of chronic experimental colitis. We hypothesized that *E. faecalis* upregulates PTS OG1RF_11616 and 12399 operons during inflammation to utilize carbohydrates present in the inflamed colon, enhance *E. faecalis* growth, and worsen colitis.

To test our hypothesis, we colonized GF WT and *Il10*$^{-/-}$ mice with *E. faecalis* (either mutant $\Delta EIIA^{11616}$ or parental *E. faecalis* OG1RF) plus the seven other bacteria in our previously-described bacteria consortium. We found no histological inflammation in WT mice regardless of bacterial strain (Figure 3.8A). There is a trend toward more histological inflammation in *Il10*$^{-/-}$ mice colonized for four weeks with the parental *E. faecalis* compared with *E. faecalis* $\Delta EIIA^{11616}$ (Figure 3.8A). The difference was statistically significant after eight weeks of colonization (Figure 3.8A). Spontaneous secretion of IL-12/23 p40 by colonic explants from these mice generally parallels the severity of histological inflammation though differences

in IL-12/23 p40 secretion between parental *E. faecalis* OG1RF and *E. faecalis* ΔEIIA^{11616} were statistically significant at four weeks after colonization (Figure 3.9). These data suggest that the presence of EIIA^{11616} in *E. faecalis* promotes colitis in this model.

Similar to the *E. faecalis* ΔEIIA^{11616} experiment, we also colonized GF WT and *Il10*$^{-/-}$ mice with *E. faecalis* (either mutant *E. faecalis* ΔEII^{gnt} or parental *E. faecalis* OG1RF) plus the seven other bacterial strains in our previously-described bacterial consortium. After five weeks of colonization, *Il10*$^{-/-}$ mice colonized with parental *E. faecalis* have significantly more histological inflammation and IL-12/23 p40 secretion from colon explants than *Il10*$^{-/-}$ mice colonized with *E. faecalis* ΔEII^{gnt} (Figure 3.10 and 3.11). However, after ten weeks of colonization, the differences in histological inflammation and IL-12/23 p40 secretion between the two groups of mice are absent (Figure 3.10 and 3.11). These data suggest that colonization with PTS EII^{gnt}-expressing *E. faecalis* accelerates the onset of experimental colitis in *Il10*$^{-/-}$ mice, but does not affect the colitis severity at later time points.

We next hypothesized that the decreased colitis severity in *Il10*$^{-/-}$ mice colonized with *E. faecalis* ΔEII^{gnt} or *E. faecalis* ΔEIIA^{11616} is due to decreased luminal survival of the mutant strains relative to the parental strain. To test this, we determined the abundance of *E. faecalis* in cecal contents from the colonized mice using *E. faecalis*-specific 16S primers and real-time qPCR. There are similar proportions of parental and mutant *E. faecalis* ΔEIIA^{11616} or *E. faecalis* ΔEII^{gnt} in the cecal contents of *Il10*$^{-/-}$ mice with colitis (Figure 3.12). This suggests that neither PTS EIIA^{11616} or PTS EII^{gnt} confer a survival advantage to luminal *E. faecalis* during inflammation. However, we observed that the relative abundances of *Bacteroides* species (*B. thetaiotaomicron* and *B. vulgatus*) are significantly decreased in *Il10*$^{-/-}$ mice colonized with parental *E. faecalis* compared with mutant *E. faecalis* ΔEIIA^{11616} or *E. faecalis* ΔEII^{gnt} (Figure

3.12). *R. gnavus* is also significantly decreased in *Il10*$^{-/-}$ mice colonized with parental *E. faecalis* compared with *E. faecalis EII*^{gnt} (Figure 3.12B). Further, we observed that the relative abundances of *B. longum* and *B. thetaiotaomicron* are significantly decreased in WT mice colonized with parental *E. faecalis* compared with mutant *E. faecalis* ΔEIIA^{11616} (Figure 3.13). These data suggest that *E. faecalis* PTS EIIA^{11616} and *E. faecalis* PTS EII^{gnt} may compete for nutrients used by *Bacteroides* species for growth and/or survival during experimental colitis development.

3.4 The pro-inflammatory potential of EIIA^{11616} requires the presence of *Bacteroides*

Our data demonstrate that *Il10*$^{-/-}$ mice colonized with a mixture of bacteria that includes PTS-deficient *E. faecalis* is associated with attenuated colitis compared with a mixture of bacteria that includes the parental *E. faecalis.* This suggests that the presence of PTS EIIA^{11616} and EII^{gnt} in *E. faecalis* promotes colitis. However, the mechanisms of how these two *E. faecalis* PTS promote experimental colitis are unclear. First, we tried to determine whether these operons enhance colitis directly through *E. faecalis* or indirectly through other bacteria. To determine whether PTS EIIA^{11616} exerts its pro-colitogenic effect independently of other bacteria, we mono-colonized GF *Il10*$^{-/-}$ mice with *E. faecalis* ΔEIIA^{11616} or parental *E. faecalis*. Since no other bacteria are present in this system, then any observed differences in inflammation would presumably be solely due to the effects of PTS EIIA^{11616} on *E. faecalis*. We chose to evaluate colonic inflammation of these mono-colonized mice at a later timepoint (20 weeks) than we did the oct-colonized mice referenced above (8-10 weeks) because the kinetics of inflammation in *E. faecalis*-monocolonized *Il10*$^{-/-}$ mice is known to be slower compared with mice mono-colonized with other bacterial strains, such as *E. coli* NC101(Kim et al., 2005). We found that PTS EIIA^{11616} is not associated with increased histological inflammation and IL-12/23 p40 secretion

in *E. faecalis* mono-colonized *Il10*$^{-/-}$ mice at 10 weeks or 20 weeks (Figure 3.14). We also found no statistically significant difference in luminal concentration of bacteria in colons of mice mono-colonized with *E. faecalis* or *E. faecalis* ΔEIIA^{11616} (Figure 3.15). Together these results indicate that the pro-inflammatory qualities of *E. faecalis* PTS EIIA^{11616} require the presence of other bacterial species. Since we previously observed that the presence of *E. faecalis* PTS EIIA^{11616} and EII^{gnt} is associated with lower numbers of *Bacteroides* species in the oct-colonized mice (Figure 3.11), we hypothesized that these two *E. faecalis* PTS operons exert their pro-colitogenic effects via *Bacteroides* species in the colon lumen. To determine whether the presence of *Bacteroides* species is necessary for the *E. faecalis* PTS EIIA^{11616}-mediated increased colitis observed in oct-colonized *Il10*$^{-/-}$ mice (Figures 3.8 and 3.9), we repeated experiments described in Figures 3.8 and 3.9, but omitted the two *Bacteroides* species from the colonizing inoculum. In the absence of the two *Bacteroides* species, we found that the presence of *E. faecalis* PTS EIIA^{11616} has no effect on the severity of histological colon inflammation or levels of IL-12/23 p40 secretion (Figure 3.16). Therefore, we conclude that *Bacteroides* species are required for *E. faecalis* PTS EIIA^{11616}-mediated colitis.

3.5 Figures

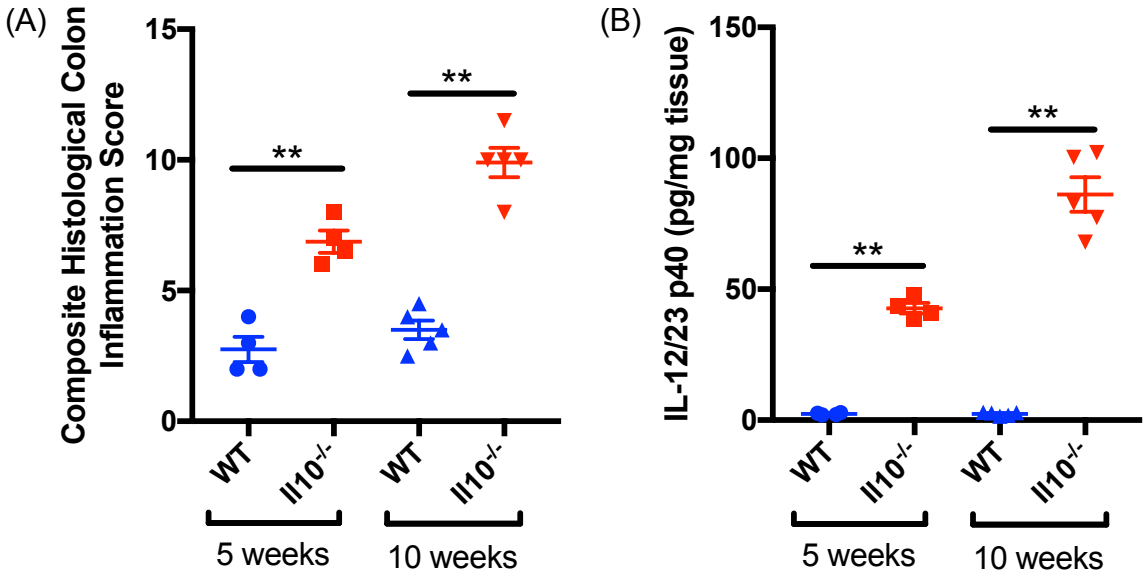

Figure 3.1 Spontaneous intestinal inflammation develops in *Il10*$^{-/-}$ mice colonized with a defined mixture of intestinal bacteria. Germ free WT and *Il10*$^{-/-}$ mice were colonized with 8 microbes (*Enterococcus faecalis*, *Ruminococcus gnavus*, *Faecalibacterium prausnitzii*, *Lactobacillus rhamnosus, Bacteroides thetaiotaomicron*, *Bacteroides vulgatus, Bifodobacterium longum, and Escherichia coli*) for 5 and 10 weeks. (A) Sum of blinded histological inflammation scores of four segments of colon from each mouse. (B) IL-12/23 p40 concentrations in culture supernatants from colonic tissue explant cultures normalized per mg of tissue. Data are presented as mean ± standard error of mean, n = 4 - 5 mice/group. ** $p < 0.01$ versus WT.

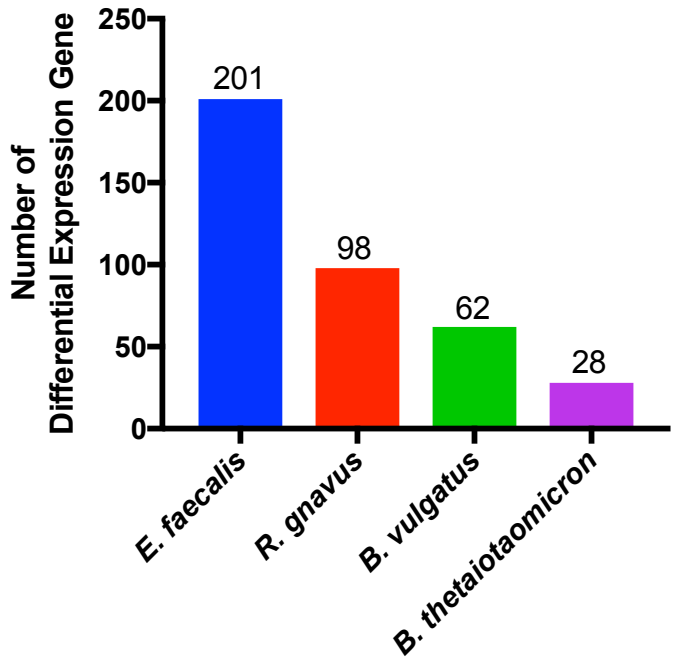

Figure 3.2 Number of differentially expressed microbial genes between WT and *Il10*$^{-/-}$ mice colonized with a defined mixture of intestinal bacteria. Bacterial RNA was isolated from cecal contents from the 10-week-colonized WT and *Il10*$^{-/-}$ mice described in the legend to Figure 3.1. Bacterial gene expression was determined using microbial RNA-seq and GeneSpring GX software. For the species that had at least one differentially expressed gene, we present the number of genes with ≥ 1.5-fold change in gene expression (WT vs. *Il10*$^{-/-}$) with an FDR-corrected p value of ≤ 0.05.

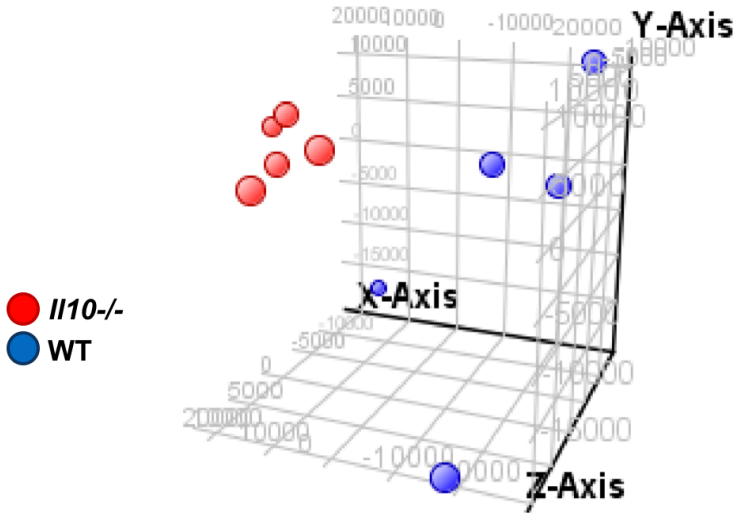

Figure 3.3 Colitis is associated with an altered luminal bacterial meta-transcriptome. Principal component analysis of the luminal bacterial gene expression profiles of ten-week-colonized WT and *Il10*$^{-/-}$ mice described in the legend to Figure 3.1.

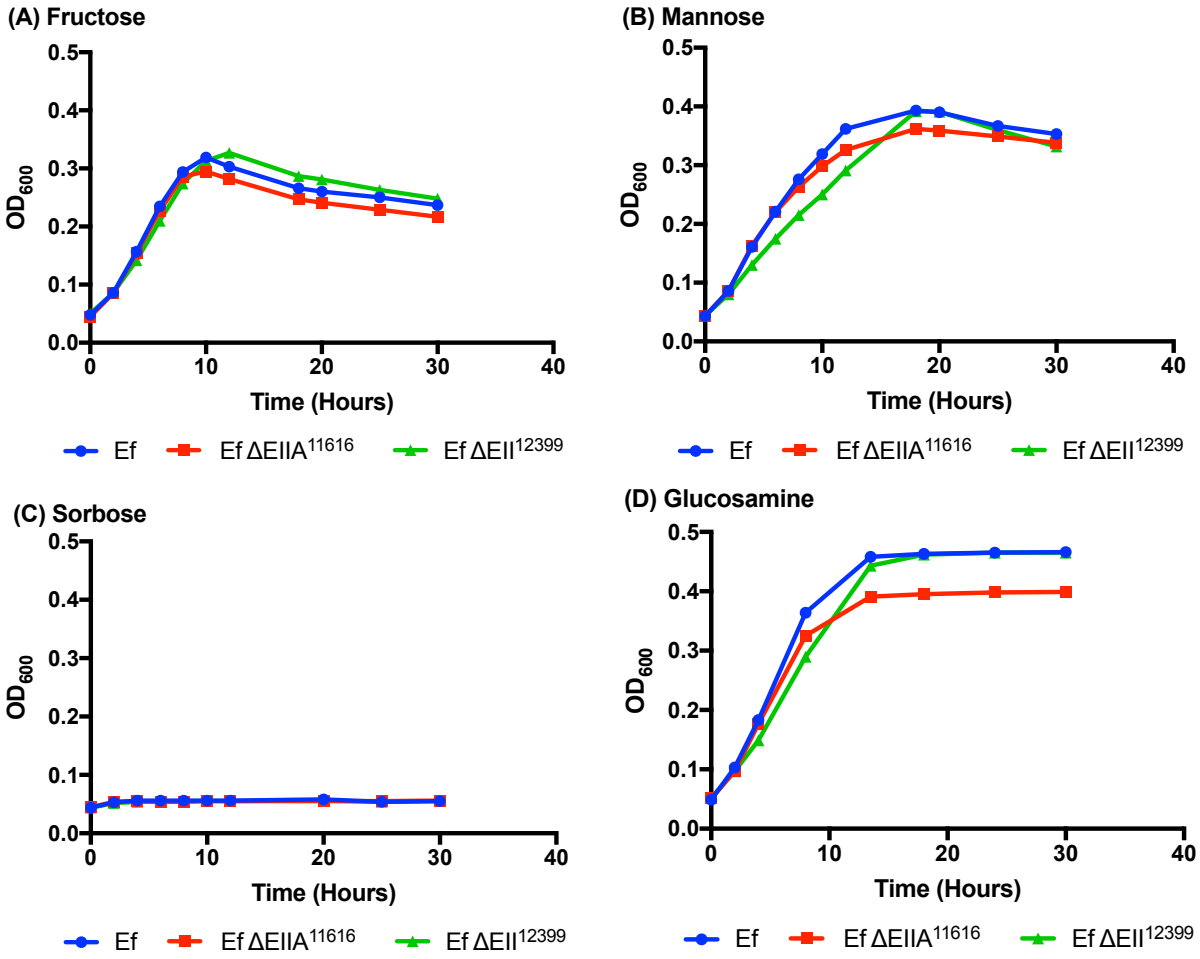

Figure 3.4 Growth curves of parental and mutant *E. faecalis* strains in minimal media supplemented with selected carbon sources. Parental *E. faecalis* (Ef), *E. faecalis* Δ EIIA^{11616} (Ef Δ EIIA^{11616}), and *E. faecalis* ΔEII^{12399} (Ef ΔEII^{12399}) were grown aerobically in minimal media supplemented with the indicated sole carbon source (A) 0.5 % fructose, (B) 0.5% mannose, (C) 0.5% sorbose, and (D) 0.4% glucosamine.

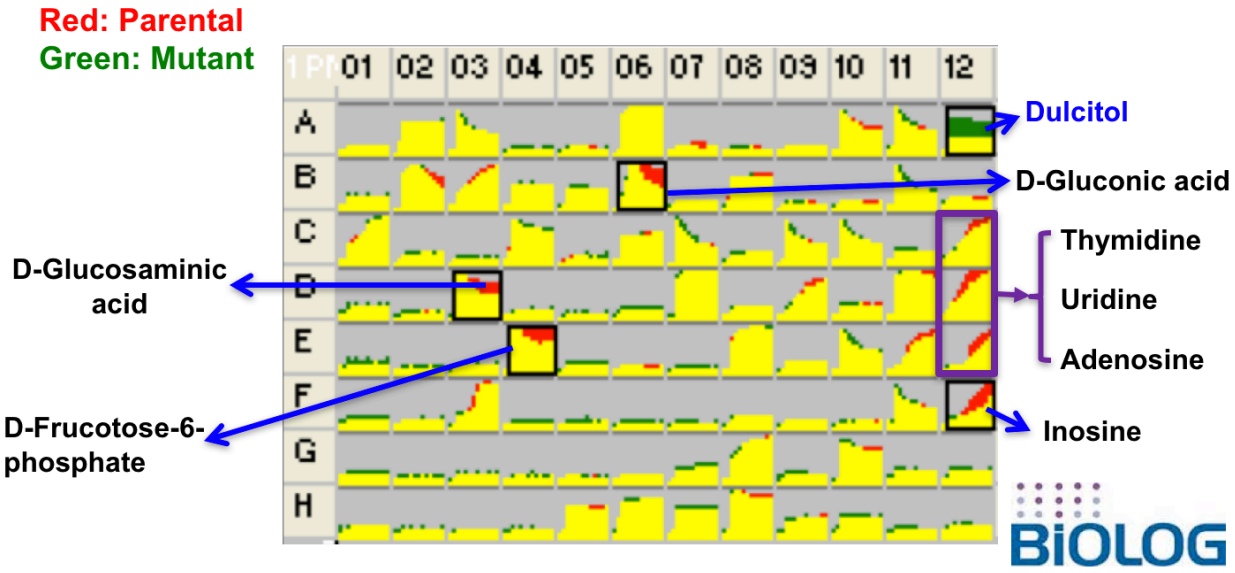

Figure 3.5 High throughput metabolic screen of *E. faecalis* and *E. faecalis* ΔEIIA^{11616} strains grown in the presence of various sole carbon sources. Output from one representative Phenotype Micro Array™ PM1, which predominantly includes carbohydrate and carboxylate substrates as carbon sources. Each well in the 96-well plate contains a unique carbon source. Each plot in the 96 cells depicted above presents dye reduction (i.e. metabolic activity) on the Y-axis and time on the X-axis (0-16 hours). Red areas represent times at which the parental strain metabolizes the carbon source better than the mutant strain and green areas represent times at which the mutant strain metabolizes the carbon source better than the parental strain. Cells with apparent differences in metabolism of the carbon source between the two strains are highlighted.

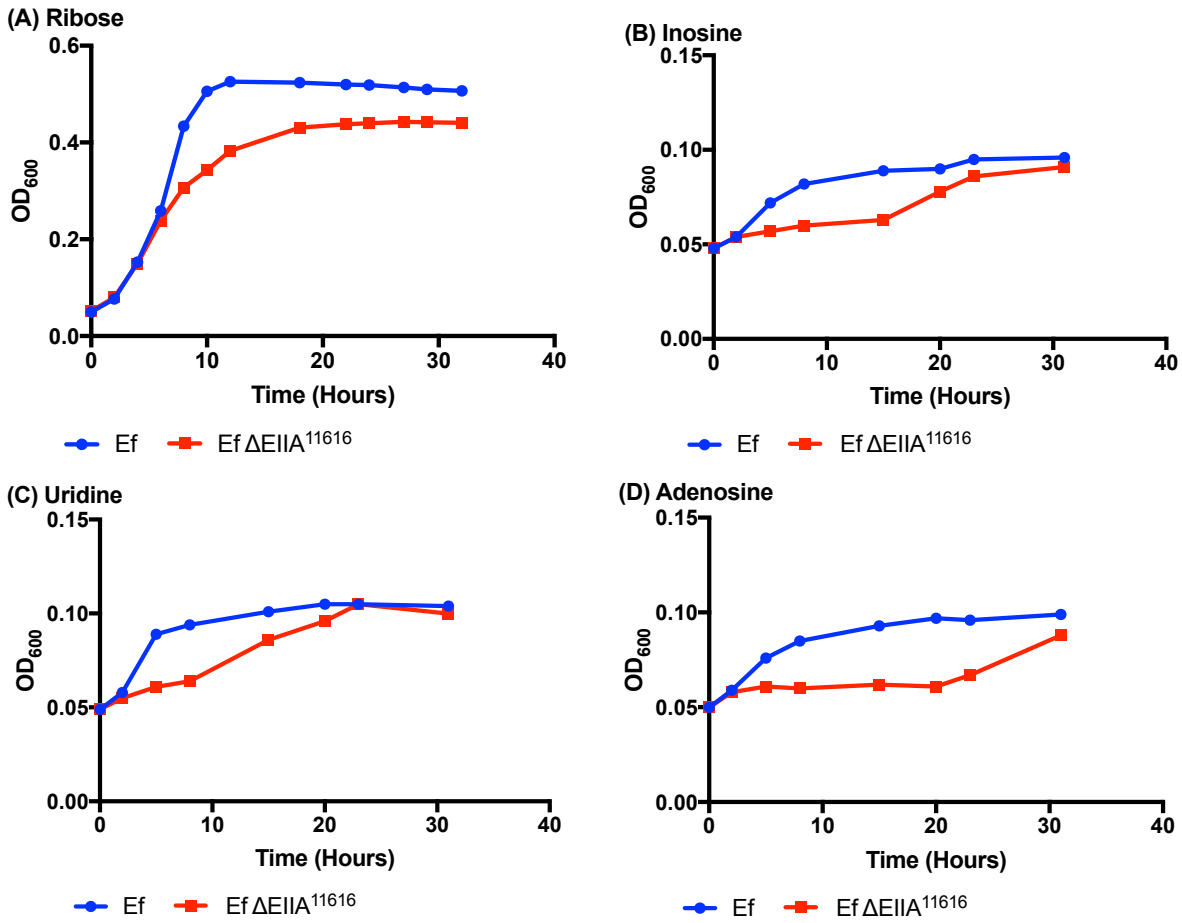

Figure 3.6 The presence of PTS EIIA^{11616} is associated with increased *E. faecalis* growth minimal media containing ribose and nucleosides as the sole carbon source. Growth curves of parental *E. faecalis* (Ef) and *E. faecalis* Δ EIIA^{11616} (Ef Δ EIIA^{11616}) grown in minimal media supplemented with the following sole carbon sources (A) 0.5 % ribose, (B) 0.5% inosine, (C) 0.5% uridine, and (D) 0.1% adenosine under aerobic conditions.

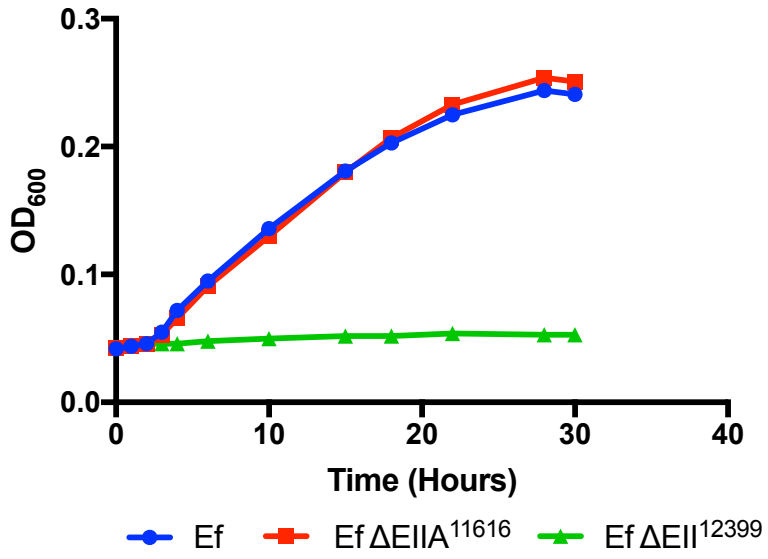

Figure 3.7 PTS EII^{12399} is essential for gluconate utilization by *E. feacalis.* Parental *E. faecalis* (Ef), *E. faecalis* Δ EIIA^{11616} (Ef Δ EIIA^{11616}), and *E. faecalis* ΔEII^{12399} (Ef ΔEII^{12399}) were grown in minimal media supplemented with sodium gluconate as the sole carbon source.

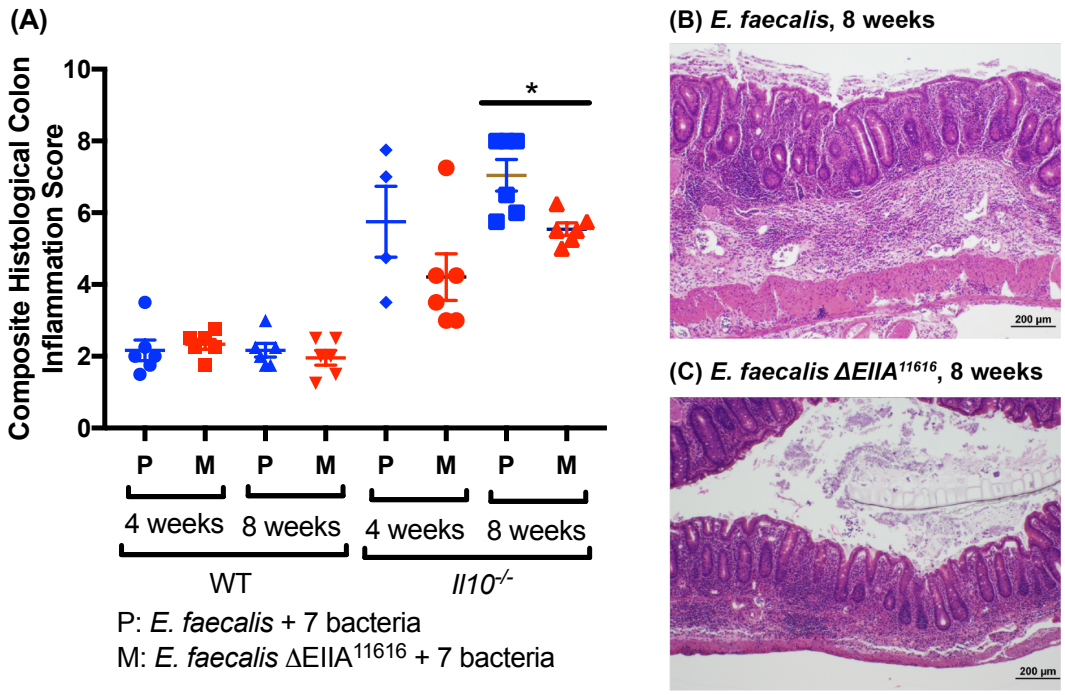

Figure 3.8 PTS EIIA^{11616} is associated with increased histological colon inflammation in *Il10*$^{-/-}$ mice. WT and *Il10*$^{-/-}$ mice were colonized with seven intestinal bacterial species identified in the legend of Figure 3.1 plus either parental *E. faecalis* or *E. faecalis* ΔEIIA^{11616} for 4 or 8 weeks. (A) Sum of blinded histological inflammation scores of four colon segments. Data are presented as mean ± standard error of mean, n = 4 - 6 mice/group. *p < 0.05 versus parental *E. faecalis* group. Representative photomicrographs of H & E stained colon tissue from *Il10*$^{-/-}$ mice colonized for eight weeks with bacterial mixture that includes (B) parental *E. faecalis* or (C) *E. faecalis* ΔEIIA^{11616}.

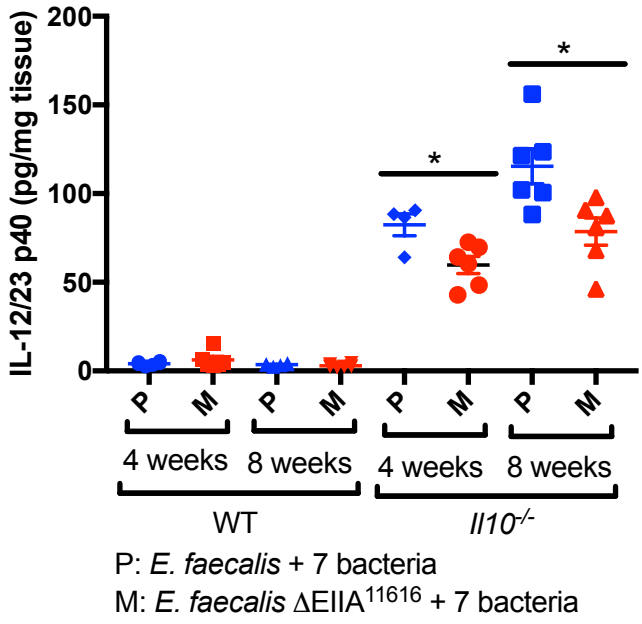

Figure 3.9 PTS EIIA^{11616} is associated with increased IL-12/23 p40 secretion by colon explants from *Il10*$^{-/-}$ mice. WT and *Il10*$^{-/-}$ mice were colonized as described in the legend to Figure 3.8. IL-12/23 p40 concentrations in colonic tissue explant culture supernatants were normalized per mg tissue weight. Data are presented as mean ± standard error of mean, n = 4 - 6 mice/group. * $p < 0.05$ versus parental *E. faecalis* group.

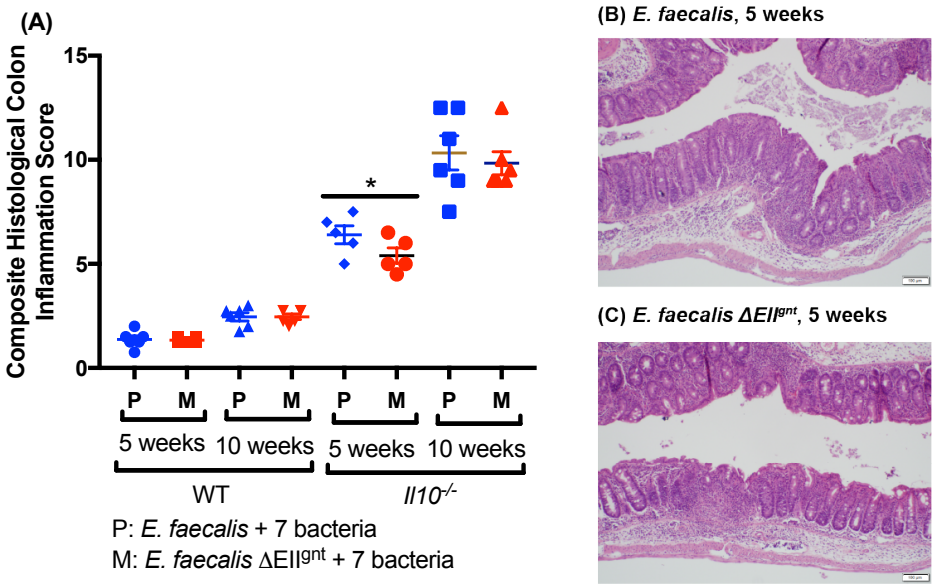

Figure 3.10 PTS EII^{gnt} is associated with increased histological colon inflammation in *Il10*$^{-/-}$ mice. WT and *Il10*$^{-/-}$ mice were colonized with seven bacterial species identified in the legend to Figure 3.1 plus either parental *E. faecalis* or *E. faecalis* ΔEII^{gnt} for five or ten weeks. (A) Sum of blinded histological inflammation scores of four colon segments. Data are presented as mean ± standard error of mean, n = 5 - 6 mice/group. * $p < 0.05$ versus parental *E. faecalis* group. Representative photomicrographs of H & E stained colon tissue from *Il10*$^{-/-}$ mice colonized for five weeks with bacterial mixture that includes (B) parental *E. faecalis* or (C) *E. faecalis* ΔEII^{gnt}.

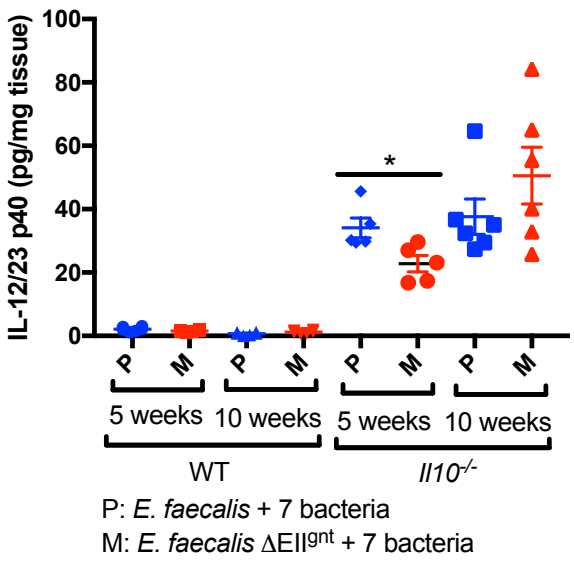

Figure 3.11 PTS EII^{gnt} is associated with increased IL-12/23 p40 secretion by colon explants from *Il10*$^{-/-}$ mice. WT and *Il10*$^{-/-}$ mice were colonized as described in the legend to Figure 3.10. IL-12/23 p40 concentrations in colonic tissue explant culture supernatants normalized per mg tissue weight. Data are presented as mean ± standard error of mean, n = 5 - 6 mice/group. * $p < 0.05$ versus parental *E. faecalis* group.

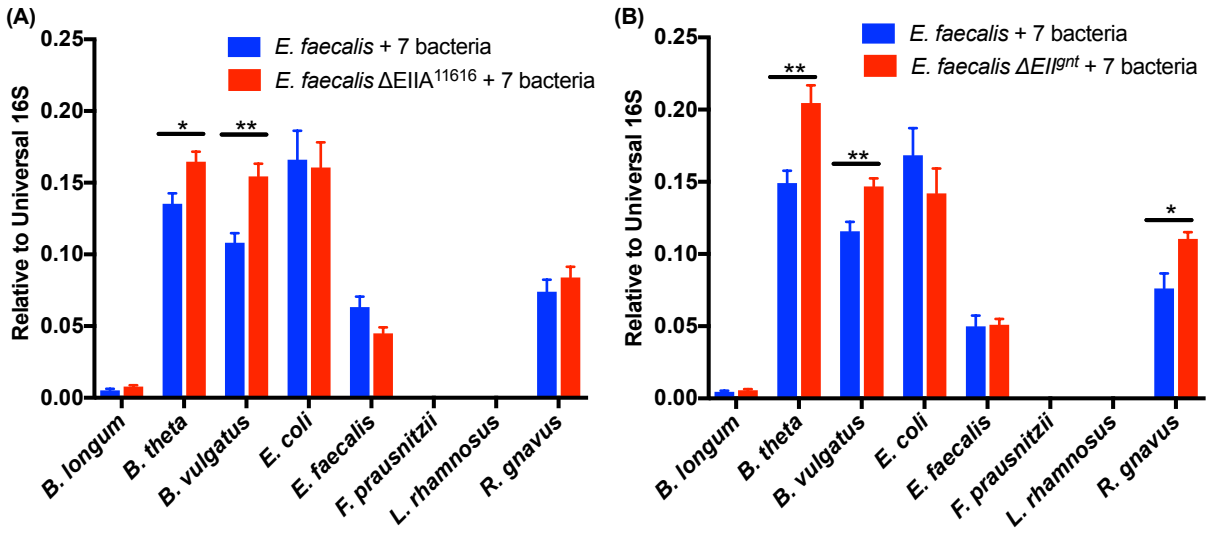

Figure 3.12 PTS EIIA^{11616} and EII^{gnt} are associated with an altered luminal bacterial composition in *Il10*$^{-/-}$ mice. (A) Relative abundance of eight bacterial species in cecal contents from *Il10*$^{-/-}$ mice colonized for eight weeks as described in the legend to Figure 3.8 as measured by real-time PCR relative to universal 16S. n = 6 mice/group. (B) Relative abundance of eight bacterial species in cecal contents from *Il10*$^{-/-}$ mice colonized for five weeks as described in the legend to Figure 3.10 as measured by real-time qPCR relative to universal 16S. n = 5 - 6 mice/group. Data are presented as mean ± standard error of mean. * $p < 0.05$ versus parental *E. faecalis* group. ** $p < 0.01$ versus parental *E. faecalis* group.

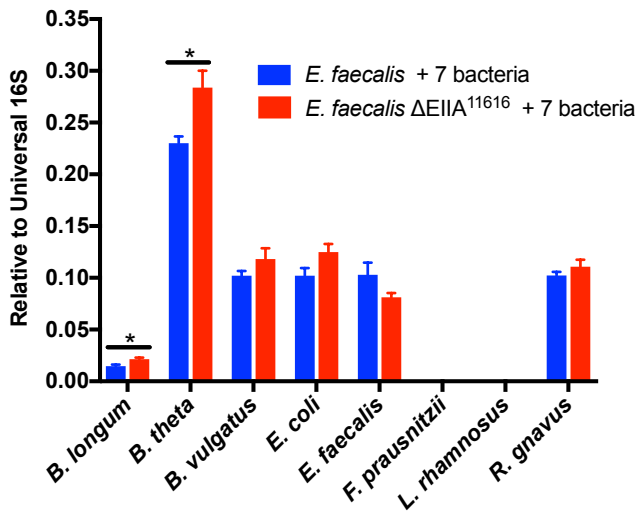

Figure 3.13 PTS EIIA^{11616} is associated with an altered luminal bacterial composition in WT mice. (A) Relative abundance of eight bacterial species in cecal contents from WT mice colonized for eight weeks as described in the legend to Figure 3.8 as measured by real-time PCR relative to universal 16S. n = 6 mice/group. Data are presented as mean ± standard error of mean. * $p < 0.05$ versus parental *E. faecalis* group.

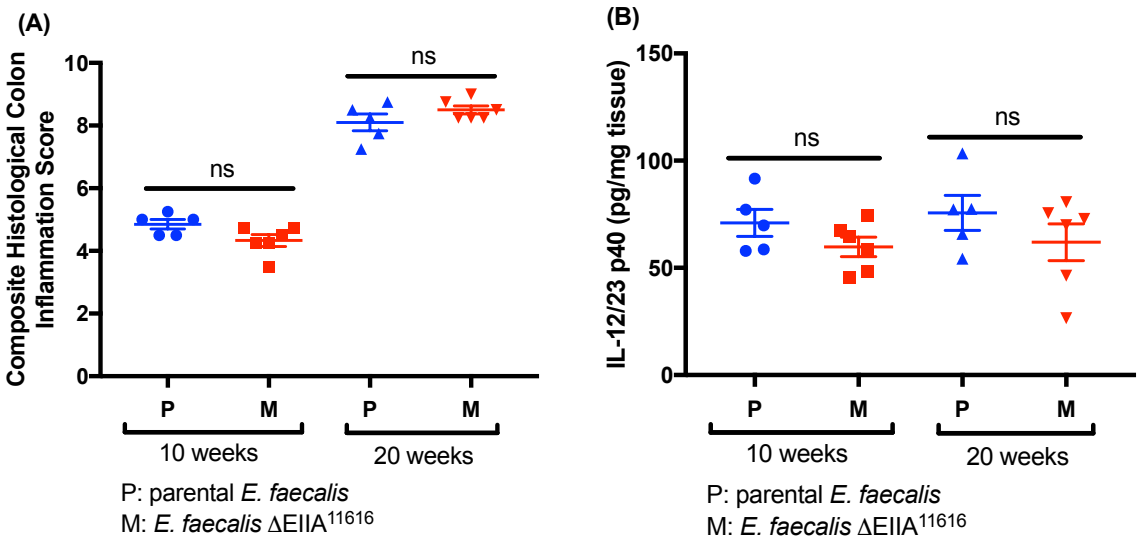

Figure 3.14 PTS EIIA^{11616} does not affect colitis severity in *E. faecalis* mono-colonized *Il10*$^{-/-}$ mice. *Il10*$^{-/-}$ mice were mono-colonized with parental *E. faecalis* or *E. faecalis* ΔEIIA^{11616} for 10 and 20 weeks. (A) Sum of blinded histological inflammation scores of four colon segments. (B) IL-12/23 p40 concentrations in colonic tissue explant culture supernatants normalized per mg tissue weight. Data are presented as mean ± standard error of mean, n = 5 - 6 mice/group, ns: not significant.

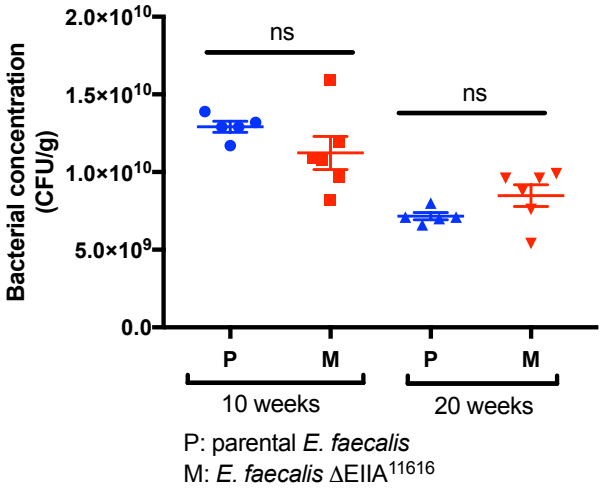

Figure 3.15 PTS EIIA^{11616} does not affect luminal *E. faecalis* concentration in *E. faecalis* mono-colonized *Il10*$^{-/-}$ mice. Luminal *E. faecalis* concentrations in *Il10*$^{-/-}$ mice mono-colonized for 10 and 20 weeks with either parental *E. faecalis* or *E. faecalis* ΔEIIA^{11616}. Data are presented as mean ± standard error of mean, n = 5 - 6 mice/group, ns: not significant.

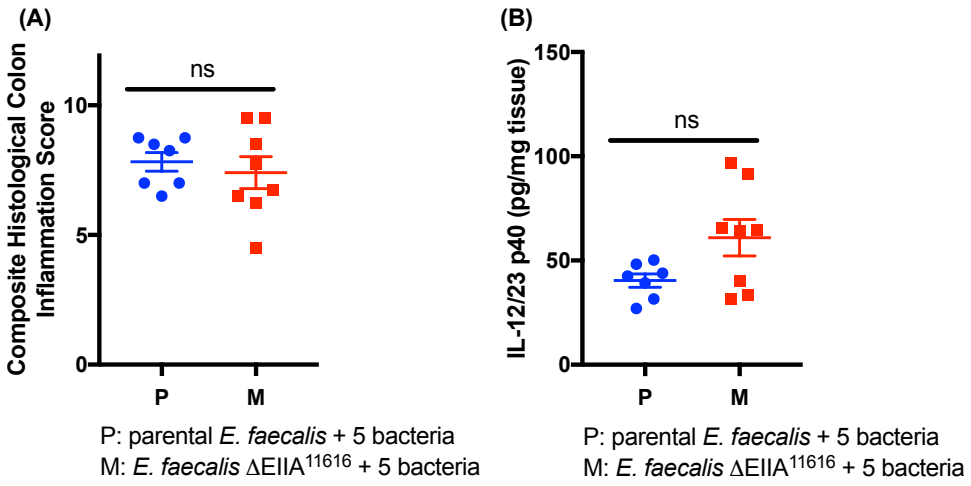

Figure 3.16 *E. faecalis* PTS EIIA^{11616} does not affect colitis severity in the absence of *Bacteroides* species. *Il10*$^{-/-}$ mice were colonized for ten weeks with parental *E. faecalis* or *E. faecalis* ΔEIIA^{11616} plus the five non-*Bacteroides* species described in the legend to Figure 3.1 (*R. gnavus*, *F. prausnitzii*, *L. rhamnosus, B. longum, and E. coli*). (A) Sum of blinded histological inflammation scores of four colon segments. (B) IL-12/23 p40 concentrations in colonic tissue explant culture supernatants normalized per mg tissue weight. Data are presented as mean ± standard error of mean, n = 7 - 8 mice/group, ns: not significant.

3.6 Tables

Table 3.1 Conserved protein domains significantly enriched in luminal bacteria from *Il10*$^{-/-}$ vs. WT mice colonized for ten weeks with a mixture of intestinal bacterial species.

Score	Conserved Domain	FDR q value
2.12	Amino-acyl tRNA Synthetase	0.001
2.05	Translation Factor-II-Like	0.002
1.92	Mur Ligase Family	0.011
1.90	Phosphoribosyl Transferase Domain	0.013
1.87	Sugar Transferase	0.015
1.86	Glycyl Radical Superfamily	0.017
1.83	PTS Regulation Domain	0.019
1.83	Cold Shock Domain	0.018
1.77	GH18 Chitinase-Like Superfamily	0.034
1.71	MutL Transducer Domain	0.053

Table 3.2 Conserved protein domains significantly enriched in luminal bacteria from WT vs. *Il10*$^{-/-}$ colonized for ten weeks with a mixture of intestinal bacterial species.

Score	Conserved Domain	FDR q value
1.92	Thioredoxin Family	0.052
1.92	ATPase Superfamily	0.040
1.79	Iron Dicitrate Transport	0.099
1.70	Inorganic Cation Transporter	0.155
1.62	Two-component Regulator Proteins	0.218
1.61	Nitro-FMN Reductase Superfamily	0.204
1.59	Pyridine Nucleotide-Disulfide Oxidoreductase	0.215

Table 3.3 Significantly upregulated genes in cecal *E. faecalis* OG1RF from *Il10*$^{-/-}$ vs. WT mice colonized with eight bacterial species for 10 weeks

Gene_ID	Fold Change	Product
OG1RF_11616	27.09	PTS family mannose/fructose/sorbose porter component IIA
OG1RF_11614	13.35	PTS family mannose/fructose/sorbose porter component IIC
OG1RF_11613	12.60	PTS family mannose/fructose/sorbose porter component IID
OG1RF_11612	11.87	phosphosugar-binding protein
OG1RF_11611	10.48	phosphosugar isomerase
OG1RF_11615	11.36	PTS family ascorbate porter, IIB component
OG1RF_11610	6.29	hypothetical protein
OG1RF_10367	5.19	decarboxylase
OG1RF_10369	4.83	Na+/H+ antiporter NhaC
OG1RF_12405	4.56	6-phosphogluconate dehydrogenase
OG1RF_12398	4.54	mannonate dehydratase
OG1RF_10368	4.61	amino acid permease
OG1RF_12403	4.11	putative glycerol dehydrogenase
OG1RF_12182	4.04	acetyl-CoA carboxylase biotin carboxyl carrier subunit
OG1RF_12402	3.67	PTS family mannose/fructose/sorbose porter component IIC
OG1RF_12404	3.51	D-isomer specific 2-hydroxyacid dehydrogenase
OG1RF_12397	3.78	2-dehydro-3-deoxyphosphogluconate aldolase/4-hydroxy-2-oxoglutarate aldolase
OG1RF_12544	3.41	sporulation initiation inhibitor protein Soj
OG1RF_12399	3.54	PTS family mannose/fructose/sorbose porter component IIA
OG1RF_10430	3.30	RpiR family phosphosugar-binding transcriptional regulator
OG1RF_12401	3.48	PTS family mannose/fructose/sorbose porter component IID
OG1RF_10851	3.68	UDP-glucose--hexose-1-phosphate uridylyltransferase
OG1RF_12080	2.91	ribosomal protein L7/L12
OG1RF_10166	3.10	50S ribosomal protein L6
OG1RF_10164	3.18	ribosomal protein S14p/S29e
OG1RF_12179	3.33	acetyl-coA carboxylase carboxyl transferase subunit beta
OG1RF_10178	3.03	50S ribosomal protein L17
OG1RF_11167	2.71	alanine--tRNA ligase
OG1RF_10165	2.98	30S ribosomal protein S8
OG1RF_10853	3.00	hypothetical protein
OG1RF_10216	2.78	6-phospho-beta-glucosidase
OG1RF_11810	2.83	amino acid permease
OG1RF_11283	2.96	chorismate synthase
OG1RF_10152	2.74	ribosomal protein L4/L1 family protein
OG1RF_12331	2.73	secreted lipase
OG1RF_10177	2.73	DNA-directed RNA polymerase subunit alpha
OG1RF_10753	2.82	6-phospho-beta-glucosidase

OG1RF_10893	2.75	phenylalanyl-tRNA synthetase alpha subunit
OG1RF_12400	2.84	PTS family mannose/fructose/sorbose porter, IIB component
OG1RF_10159	2.56	50S ribosomal protein L29
OG1RF_10217	2.58	phosphoglycerate mutase
OG1RF_11875	2.55	acyl-CoA thioester hydrolase
OG1RF_10559	2.60	BglG family transcriptional antiterminator
OG1RF_10175	2.52	30S ribosomal protein S13
OG1RF_11938	2.48	fumarate reductase
OG1RF_10281	2.38	secreted antigen
OG1RF_10162	2.45	50S ribosomal protein L24
OG1RF_10638	2.41	ABC superfamily ATP binding cassette transporter, ABC protein
OG1RF_10894	2.47	phenylalanyl-tRNA synthetase beta subunit
OG1RF_12406	2.47	RpiR family phosphosugar-binding transcriptional regulator
OG1RF_10637	2.44	oligopeptide ABC superfamily ATP binding cassette transporter, membrane protein
OG1RF_10660	2.54	ATP-dependent DNA helicase
OG1RF_10208	2.38	hypoxanthine phosphoribosyltransferase
OG1RF_10636	2.41	oligopeptide ABC superfamily ATP binding cassette transporter, membrane protein
OG1RF_10084	2.45	cell wall surface anchor protein
OG1RF_11285	2.42	3-phosphoshikimate 1-carboxyvinyltransferase
OG1RF_10736	2.23	isoleucine--tRNA ligase
OG1RF_10752	2.38	PTS family lactose/cellobiose (lac) porter component IIC
OG1RF_10147	2.26	elongation factor EF1A
OG1RF_10671	2.33	extracellular protein
OG1RF_11839	2.19	glycyl-tRNA synthetase beta subunit
OG1RF_10807	2.19	glutathione S-transferase
OG1RF_11330	2.26	DNA topoisomerase (ATP-hydrolyzing) subunit A
OG1RF_12180	2.33	acetyl-CoA carboxylase subunit A
OG1RF_12231	2.21	valine--tRNA ligase
OG1RF_10286	2.22	membrane protein
OG1RF_11022	2.12	ABC superfamily ATP binding cassette transporter, binding protein
OG1RF_11840	2.13	glycyl-tRNA synthetase alpha subunit
OG1RF_12543	2.15	plasmid partition ParB protein
OG1RF_12311	2.07	peptide ABC superfamily ATP binding cassette transporter, binding protein
OG1RF_10657	2.08	methionine--tRNA ligase
OG1RF_10207	2.07	tRNA(Ile)-lysidine synthase (tRNA(Ile)-lysidinesynthetase)
OG1RF_11602	2.03	putative calcium-transporting ATPase
OG1RF_11607	1.95	membrane-oligosaccharide glycerophosphotransferase
OG1RF_11714	1.98	group 2 glycosyl transferase
OG1RF_12322	2.05	hypothetical protein

OG1RF_10452	1.86	trigger factor
OG1RF_12485	1.97	phosphatidylglycerol--membrane-oligosaccharide glycerophosphotransferase
OG1RF_12541	1.89	GTP-binding protein YchF
OG1RF_11573	1.90	UDP-N-acetylmuramate--L-alanine ligase
OG1RF_11715	1.87	glycosyltransferase
OG1RF_11818	1.78	proline--tRNA ligase
OG1RF_10337	1.79	AMP-binding family protein
OG1RF_11718	1.82	hypothetical protein
OG1RF_12385	1.73	serine/threonine protein phosphatase 1
OG1RF_10142	1.75	resolvase family site-specific recombinase
OG1RF_12493	1.76	DNA-directed RNA polymerase subunit beta
OG1RF_12141	1.73	galactose-1-phosphate uridylyltransferase
OG1RF_11667	1.74	AraC family transcriptional regulator
OG1RF_11136	1.74	neopullulanase
OG1RF_10115	1.74	GMP synthase (glutamine-hydrolyzing)
OG1RF_11711	1.73	CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase
OG1RF_11192	1.70	DNA mismatch repair protein MutS
OG1RF_12150	1.68	5-formyltetrahydrofolate cyclo-ligase
OG1RF_11694	1.67	ABC superfamily ATP binding cassette transporter, ABC protein
OG1RF_10714	1.64	exodeoxyribonuclease VII small subunit
OG1RF_11417	1.63	aminotransferase
OG1RF_10001	1.60	ATPase
OG1RF_10958	1.64	Ser/Thr protein phosphatase
OG1RF_10923	1.63	DNA-directed RNA polymerase sigma subunit RpoE
OG1RF_11724	1.58	group 2 glycosyl transferase
OG1RF_10459	1.60	NAD-dependent DNA ligase LigA
OG1RF_11713	1.63	CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase
OG1RF_11449	1.59	tyrosine--tRNA ligase
OG1RF_11300	1.56	transketolase
OG1RF_11819	1.57	RIP metalloprotease RseP
OG1RF_11977	1.55	transcription antiterminator LicT
OG1RF_10212	1.54	lysine--tRNA ligase
OG1RF_12528	1.56	U32 family peptidase
OG1RF_11731	1.54	dTDP-4-dehydrorhamnose reductase
OG1RF_10209	1.54	cell division protein FtsH
OG1RF_10257	1.52	septation ring formation regulator EzrA
OG1RF_10002	1.51	DNA-directed DNA polymerase III beta subunit
OG1RF_11467	1.52	ABC superfamily ATP binding cassette transporter, ABC protein
OG1RF_10184	1.52	ABC superfamily ATP binding cassette transporter, membrane protein

Table 3.4 Significantly down-regulated genes in cecal *E. faecalis* OG1RF from *Il10*$^{-/-}$ vs. WT mice colonized with eight bacterial species for 10 weeks

Gene_ID	Fold Change	Product
OG1RF_11321	4.85	hypothetical protein
OG1RF_12506	3.40	cell wall surface anchor family protein
OG1RF_10861	3.52	hypothetical protein
OG1RF_12156	3.16	hypothetical protein
OG1RF_10317	3.21	TRAP-T family tripartite ATP-independent periplasmic transporter, hembrane protein
OG1RF_10278	3.03	N-isopropylammelide isopropylaminohydrolase
OG1RF_10277	3.05	UIT9 family protein
OG1RF_10541	2.93	transglycosylase-associated protein
OG1RF_10834	3.11	hypothetical protein
OG1RF_10396	2.80	hypothetical protein
OG1RF_11643	2.85	universal stress protein
OG1RF_11140	2.76	magnesium-importing ATPase
OG1RF_10073	2.69	transglycosylase associated protein
OG1RF_12499	2.73	chitin binding protein
OG1RF_10755	2.70	hypothetical protein
OG1RF_10599	2.86	cro/CI family transcriptional regulator
OG1RF_11929	2.82	hypothetical protein
OG1RF_10273	2.76	major facilitator family transporter
OG1RF_10276	2.67	ureidoglycolate dehydrogenase
OG1RF_10259	2.72	response regulator
OG1RF_11077	2.63	co-chaperone GrpE
OG1RF_10072	2.53	stress response regulator Gls24
OG1RF_11076	2.57	heat-inducible transcription repressor HrcA
OG1RF_11180	2.51	hypothetical protein
OG1RF_10845	2.44	VOC metalloenzyme family protein
OG1RF_11163	2.64	hypothetical protein
OG1RF_10274	2.50	carbamate kinase
OG1RF_10272	2.50	hypothetical protein
OG1RF_10268	2.38	hypothetical protein
OG1RF_10862	2.37	hypothetical protein
OG1RF_12016	2.34	diacylglycerol kinase catalytic domain protein
OG1RF_11437	2.41	cold shock protein CspA
OG1RF_11383	2.29	putative NADPH:quinone reductase
OG1RF_10069	2.29	hypothetical protein
OG1RF_10070	2.29	hypothetical protein
OG1RF_10820	2.31	LytR family response regulator
OG1RF_10090	2.37	putative lipoprotein

OG1RF_10071	2.25	stress response regulator Gls24
OG1RF_11409	2.25	NADPH-dependent FMN reductase domain protein
OG1RF_12346	2.19	cell wall surface anchor family protein
OG1RF_10003	2.25	S4 domain protein YaaA
OG1RF_10068	2.15	3-oxoacyl-[acyl-carrier-protein] reductase
OG1RF_10375	2.14	hypothetical protein
OG1RF_10025	2.13	phosphosugar-binding transcriptional regulator
OG1RF_11324	2.18	cardiolipin synthetase
OG1RF_10952	2.11	hypothetical protein
OG1RF_12324	2.00	PTT family thiamin transporter
OG1RF_11506	2.03	putative lipoprotein
OG1RF_10986	1.98	alpha-acetolactate decarboxylase
OG1RF_10271	2.00	hypothetical protein
OG1RF_12305	2.04	serine protease HtrA
OG1RF_10338	1.94	organic hydroperoxide resistance protein
OG1RF_10775	1.98	drug:H+ antiporter-1 family protein
OG1RF_12070	1.89	hypothetical protein
OG1RF_11235	1.93	PTS family porter component IIA
OG1RF_12494	1.89	ChpA/MazF transcriptional modulator
OG1RF_10720	1.85	putative lipoprotein
OG1RF_11303	1.86	cysteine synthase
OG1RF_11505	1.84	hypothetical protein
OG1RF_11246	1.83	glyceraldehyde-3-phosphate dehydrogenase
OG1RF_12360	1.82	lactoylglutathione lyase
OG1RF_12488	1.81	DNA starvation/stationary phase protection protein Dps
OG1RF_10632	1.80	pentapeptide repeat family protein
OG1RF_10359	1.83	ferrous iron transport protein A
OG1RF_11836	1.84	acylphosphatase
OG1RF_10540	1.79	oligopeptide ABC superfamily ATP binding cassette transporter, binding rotein
OG1RF_11745	1.75	protein of hypothetical function DUF1696
OG1RF_10117	1.72	lipase/acylhydrolase
OG1RF_12047	1.78	regulatory protein Spx
OG1RF_10666	1.71	methylglyoxal synthase
OG1RF_10049	1.70	Veg protein
OG1RF_10085	1.73	diacylglycerol kinase catalytic domain protein
OG1RF_10218	1.75	thioesterase
OG1RF_10603	1.71	P-ATPase superfamily P-type ATPase copper (Cu) transporter
OG1RF_10985	1.68	acetolactate synthase
OG1RF_10237	1.65	riboflavin biosynthesis protein RibD domain protein
OG1RF_11222	1.67	family S9 peptidase
OG1RF_11080	1.68	chaperone DnaJ

OG1RF_11379	1.63	short chain dehydrogenase
OG1RF_12337	1.65	hydrolase
OG1RF_10140	1.60	putative alcohol dehydrogenase (NADP(+))
OG1RF_11981	1.59	PTS family glucitol/sorbitol porter component IIA
OG1RF_12046	1.57	adapter protein MecA
OG1RF_12228	1.58	tetrahydrofolate synthase
OG1RF_11999	1.57	P-ATPase superfamily P-type ATPase cadmium transporter
OG1RF_12232	1.52	thiol peroxidase

CHAPTER 4: DISCUSSION

4.1 Colitis-associated factors influence intestinal microbial composition and transcription

Although intestinal microbiota likely play a role in the development of human IBDs, direct evidence that microbes cause human IBDs is difficult to obtain using cross sectional studies of IBD patients. Animal models are important tools for studying how microbes influence the development of experimental colitis in particular and for understanding the pathogenesis of human IBDs in general. However, in conventionally housed animals, their intestinal microbiota is too complex to precisely define and manipulate component species and strains. On the other hand, mono- or dual- colonized animals have an overly-simplified microbiota that poorly represents the natural gastrointestinal microbial environment. In the current studies, we report using a novel simplified, defined (i.e. composed of well-characterized strain isolates with sequenced genomes) microbial consortium to study microbe-host and microbe-microbe interactions. Because we use defined microbes, we can more easily track and interpret bacterial transcriptome profiles.

Others have previously shown that experimental colitis is associated with altered luminal bacterial transcriptomes in *E. faecalis* mono-colonized mice. For example, Ocvirk et al. reported that *E. faecalis* virulence genes, *sprE* (OG1RF_11525), *gelE* (OG1RF_11526), and *fsr*-locus (OG1RF_11527 ~ 11529), are upregulated in *Il10*$^{-/-}$ mice mono-colonized with *E. faecalis* for 16 weeks (Ocvirk et al., 2015). However, these genes were not upregulated in *E. faecalis* from mice with colitis in our experiments. This discrepancy might be due to differences in microbial

complexity (mono-association vs. oct-association), observation time (16 weeks vs 10 weeks), or sampling location (colonic content vs. cecal content). Future studies are needed to determine how the presence of other bacterial species impacts the *E. faecalis* transcriptome during colitis.

Another transcriptome analysis of *E. faecalis,* but in non-inflamed mice, was reported by Lindenstrauß et al. They used microbial RNA-seq to identify potential adaptive fitness genes in *E. faecalis* OG1RF during colonization of the mouse intestinal tract (Lindenstrauß et al., 2014). They found 124 differentially expressed genes in luminal *E. faecalis* from mono-colonized WT 129 SvEv mice from Taconic compared with *E. faecalis* growing in exponential phase in BHI broth. These 124 microbial genes are involved in energy metabolism, transport and binding mechanisms, and fatty acid metabolism. Interestingly, they found that PTS EII^{gnt} and PTS $EIIB^{11615}$ are up-regulated in *E. faecalis* in the mouse intestinal tract vs. BHI broth. This suggests that *E. faecalis* adapts to the environment of the non-inflamed colon by upregulating transcription of these PTS operons and that the presence of colitis induces additional upregulation. The factors responsible for upregulation of these PTS operons in normal and inflamed colons remain to be determined, but may include specific dietary or host-derived carbohydrates. Others have shown that mouse- rather than diet-derived gluconate facilitates *E. coli* colonization of the mouse intestine (Sweeney, Laux, & Cohen, 1996). We speculate that colitis reduces concentrations of preferred *E. faecalis* carbon/energy sources in the colon lumen thus forcing *E. faecalis* to use gluconate.

We and others have also studied the effects of colitis on gene transcription in *E. coli* mono-colonized mice. We found 64 up-regulated and 150 down-regulated genes in *E. coli* NC101 from *Il10*$^{-/-}$ compared with WT mice (Patwa et al., 2011). Many of the most highly up-regulated genes encode stress response proteins such as acid tolerance protiens or heat shock

proteins that protect bacteria from reactive oxygen species. Also, genes that encode metabolism proteins such as glycerol-3phophate dehydrogenase are upregulated. In another study by Arthur et al., they found that *E. coli* NC101 from *Il10*$^{-/-}$ mice mono-colonized for 2, 12 and 20 weeks had a number of differentially expressed genes at 12 vs. 2 weeks, though the number was lower than what we had previously reported in WT vs. *Il10*$^{-/-}$ mice at ten weeks (Arthur et al., 2014). They observed no differentially expressed genes in *E. coli* from mice colonized for 20 vs. 2 weeks. The discrepancy between these results and those from Patwa et al. could be due to differences in sample collection (cecal content vs. feces), colonization time (10 weeks vs. 2, 12, and 20 weeks), control group (WT vs. *Il10*$^{-/-}$; *Rag2*$^{-/-}$), and additional treatment (AOM). In the current studies, we did not detect any genes that were significantly differentially expressed in *E. coli* from ceca of WT vs. *Il10*$^{-/-}$ mice colonized for ten weeks with eight bacterial species. The discrepancy between the current *E. coli* transcriptome data and those we published previously in mono-colonized WT and *Il10*$^{-/-}$ mice is likely due to the presence of a more complex microbiota in the current studies. Taken together, these studies indicate that various factors including inflammation, diet, time, and presence of multiple bacterial species affect microbial transcriptome profiles in intestinal bacteria from mice.

4.2 *E. faecalis* PTS EII^{pnt} and EII^{11616}

Although the *E. faecalis* gluconate PTS was already discovered in 1982 by Bernsmann et al. by a protein biochemical approach (Bernsmann, Alpert, Muss, Deutscher, & Hengstenberg, 1982), the genes for the gluconate PTS in *E. faecalis* had not been identified until 2009 (Brockmeier et al., 2009; Reinelt et al., 2009). Reinelt and Scheffek et al. reported the crystal structure of *E. faecalis* PTS EIIA^{gnt} and showed a tightly interacting dimer of EIIA^{gnt} (Reinelt et al., 2009). They also used homology modeling to suggest phosphoryl transfer reactions occur

between *E. faecalis* HPr, EIIA^{gnt} and EIIB^{gnt} components similar to *E. coli* mannose PTS components (Williams, Cai, Suh, Peterkofsky, & Clore, 2005). Lastly, they demonstrated direct interaction and phosphoryl group transfer between EIIA^{gnt} and EIIB^{gnt} components using a biochemical approach (Brockmeier et al., 2009). However, the current studies are the first report that demonstrates using genetic approaches that *E. faecalis* PTS EIIA^{gnt} is essential for *E. faecalis* growth in media where gluconate is the sole carbon source.

In addition to permitting gluconate utilization, *E. faecalis* PTS EIIA^{gnt} may also affect *E. faecalis* virulence. Recently, Peng et al. reported two *E. faecalis* PTS EII loci that regulate stress responses such as antioxidant or catabolite repression (Peng et al., 2017). They generated two PTS EIICD mutants in *E. faecalis* (Δ*pts1* and Δ*pts2*) and found that both mutant PTS strains had decreased intra-cellular survival in the J774 macrophage cell line. We have determined that the nucleotide sequence of their *E. faecalis pts1* is highly similar to that of *E. faecalis* ΔEII^{gnt}. Therefore, it is plausible that *E. faecalis* PTS EIIA^{gnt} may accentuate colitis in the presence of other bacterial species by increasing survival of *E. faecalis* in macrophages of the intestinal lamina propria. This concept is supported by work from Hondrop et al. indicating that a group A *Streptococcus* PTS regulates bacterial virulence in skin infections (Hondorp et al., 2013).

Unfortunately, we have not yet identified the substrate for the PTS encoded by the PTS EIIA^{11616} operon; however, we have determined that *E. faecalis* PTS ΔEIIA^{11616} has a slight growth defect in minimal media supplemented with glucosamine, nucleosides, or ribose. Interestingly, concentrations of ribose are increased in feces from Winnie mice, which have a mutation in the Muc2 gene that results in spontaneous colitis, as compared to C57BL/6 mice (Robinson et al., 2016). These data suggest that colitis in general may be associated with increased luminal ribose concentrations and could explain why *E. faecalis* in mice with colitis

upregulate transcription of PTS EIIA^{11616}. However, since *E. faecalis* PTS ΔEIIA^{11616} exhibit only a partial growth defect in ribose-containing minimal media, *E. faecalis* may have multiple pathways or PTS that facilitate ribose utilization. Genetically or pharmacologically inhibiting all of these ribose utilization pathways could potentially attenuate colitis more profoundly than what we observed in *E. faecalis* PTS ΔEIIA^{11616} colonized mice. Also, our *E. faecalis* PTS ΔEIIA^{11616} lacks only one gene of the entire PTS operon, EIIA^{11616} (OG1RF_11616), which may not completely abolish the function of this PTS. In this case, EIIA subunits from other PTS operons may functionally compensate for the lack of PTS EIIA^{11616}, though we think this is unlikely based on our current understanding of PTS biology in general. Perhaps deleting the entire OG1RF_11616-cointaining PTS operon in *E. faecalis* OG1RF would permit us to more definitively identify the substrate. Once we identify the substrate of PTS EIIA^{11616}, we will be better equipped to explore mechanisms by which this PTS affects colitis.

We recognize that the attenuated colitis we observed in mice colonized with *E. faecalis* PTS ΔEIIA^{11616} or *E. faecalis* ΔEII^{gnt} could be due to changes in other genes/gene expression. For instance, random mutations may have arisen in other loci during the generation of the mutant strains. Also, the attenuated colitis could be due to polar effects of the gene deletions on neighboring genes. In order to exclude these possibilities, we will need to generate genetic complemented strains of *E. faecalis* ΔEIIA^{11616} and *E. faecalis* ΔEII^{gnt} and show that these complemented strains no longer affect the colitis phenotype.

4.3 The role of *Bacteroides* in the development of *E. faecalis* PTS-mediated colitis

The presence of EIIA^{11616} or EII^{gnt}-expressing *E. faecalis* is associated with increased colitis and decreased relative abundance of two *Bacteroides* species in our oct-colonized mouse experiments. This suggests that *Bacteroides* species may attenuate colitis in this animal model

system. Others has previously shown that *Bacteroides fragilis* polysaccharide A (PSA) is immuno-regulatory and increases regulatory T cell function to prevent colitis development (Surana & Kasper, 2012). Mono-colonization of *Il10*$^{-/-}$ mice with *Bacteroides vulgatus* does not induce colitis (Sellon et al., 1998).

Still other reports suggest a pro-inflammatory role of *Bacteroides* species. Mono-colonization of HLA-B27 transgenic rats with *Bacteroides vulgatus* induces colitis (Rath, Wilson, & Sartor, 1999). *Bacteroides thetaiotaomicron* (*B. theta*) is associated with worse colitis in CD4-dnTgfb2; *Il10rb*$^{-/-}$ mice (Benjdia & Berteau, 2016; Hickey et al., 2015). However, we do not know *B. theta*'s role in the *Il10*$^{-/-}$ mouse colitis model.

To summarize, there are conflicting reports about the effects of *Bacteroides* species on experimental colitis. The type of animal model, the presence of other bacterial species, and length of colonization may explain some of the variability. We have found that *Il10*$^{-/-}$ mice mono-colonized with *E. faecalis* vs. tri-colonized with *E. faecalis* + *B. vulgatus* + *B. theta* have similar degrees of colitis (data not shown). Therefore, if *B. vulgatus* and *B. theta* attenuate colitis in *Il10*$^{-/-}$ mice, the presence of yet other bacterial species from our mixture of eight species is required. To determine this, we would need to perform additional experiments in which mice are selectively colonized with various combinations of the eight bacteria.

CHAPTER 5: CONCLUSIONS AND FUTURE PERSPECTIVES

Cross-sectional epidemiological studies have shown that dysbiosis of the intestinal microbiota is associated with IBDs. Our findings demonstrate that *E. faecalis* PTS impact the ecological structure of the intestinal microbiome through interactions with other bacteria. We also show that adaptive responses of gut bacteria to host inflammation, including upregulation of transcription of certain PTS, impacts the pathogenesis of experimental colitis.

This work lays the foundation for studies that could help to improve our understanding of host-microbial-environmental interactions in the intestine and that could lead to the development of safe and effective oral, luminally active IBD therapies that selectively target bacterial pathways.

Future studies should characterize detailed mechanisms of how *E. faecalis* PTS participate in the development of colitis. For example, what is the substrate of PTS EII^{11616} and how do it and gluconate affect IBDs in animals and humans? How does intestinal inflammation affect the concentrations of the substrates in the intestine? What is the source of substrates in the inflamed intestine? Does modifying luminal concentrations of these PTS substrates impact intestinal inflammation? Do these PTS systems affect virulence mechanisms of *E. faecalis* or other bacteria? What is the effect of global PTS regulation in *E. faecalis* on colitis? Could novel bacteriophages be engineered to selectively deliver CRISPR-Cas9 machinery to intestinal bacteria and delete specific genes such as *E. faecalis* PTS genes as an IBD therapy?

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